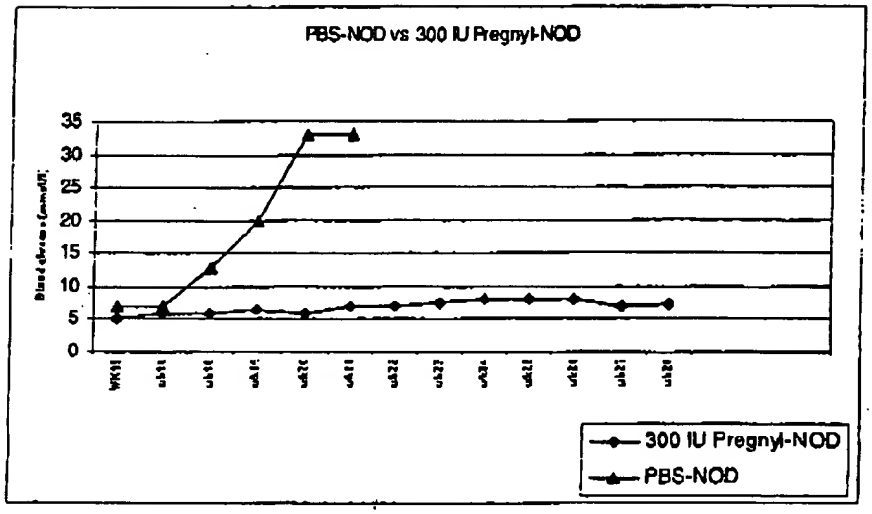

PBS-NOD vs 300 IU Pregnyl-NOD
35
30
25
20
15
10
5
0
300 IU Pregnyl-NOD
PBS-NOD

Figure 1.

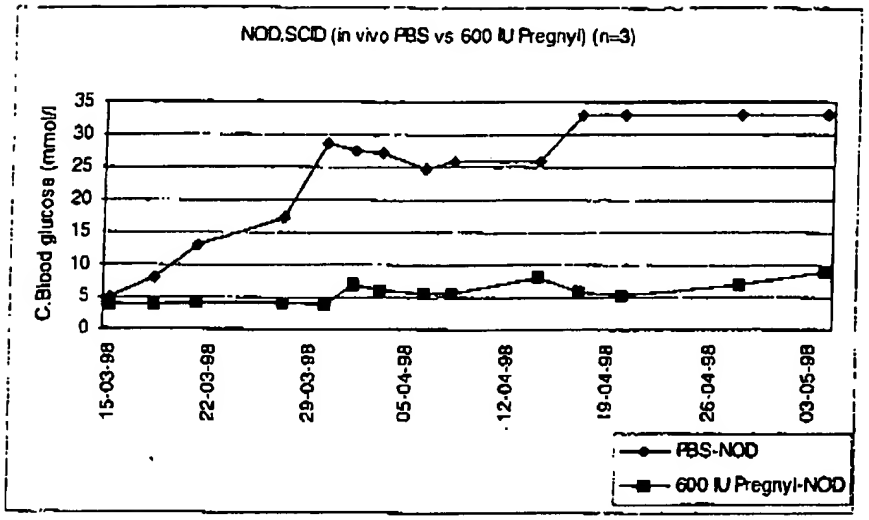

NOD.SCID (in vivo PBS vs 600 IU Pregnyl) (n=3)
C.Blood glucose (mmol/l
35
30
25
20
15
10
5
0
15-03-98
22-03-98
29-03-98
05-04-98
12-04-98
19-04-98
26-04-98
03-05-98
PBS-NOD
600 IU Pregnyl-NOD

Figure 2.

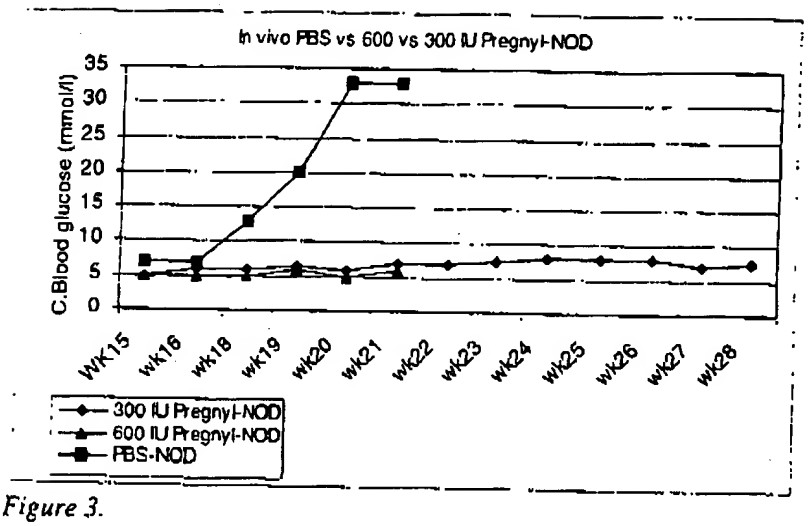

In vivo PBS vs 600 vs 300 IU Pregnyl-NOD
C.Blood glucose (mmol/l)
35
30
25
20
15
10
5
0
WK15
wk16
wk18
wk19
wk20
wk21
wk22
wk23
wk24
wk25
wk26
wk27
wk28
300 IU Pregnyl-NOD
600 IU Pregnyl-NOD
PBS-NOD

Figure 3.

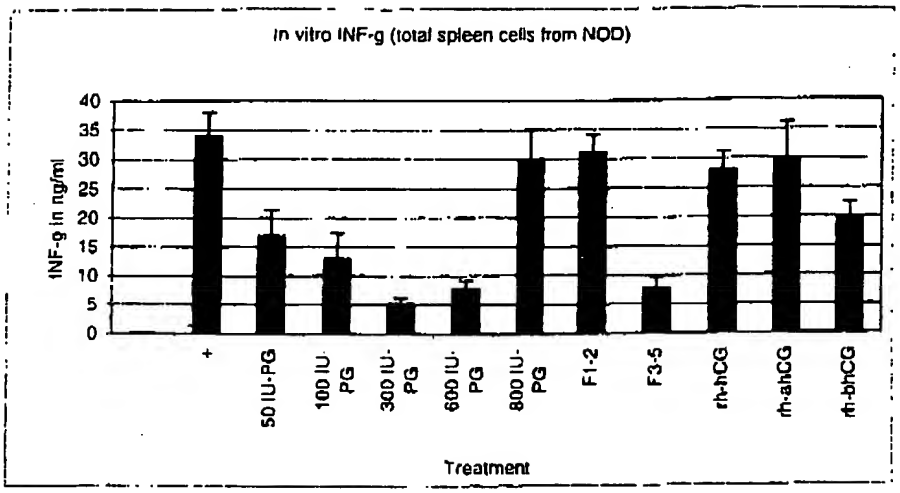

In vitro INF-g (total spleen cells from NOD)
INF-g in ng/ml
40
35
30
25
20
15
10
5
0
+
50 IU-PG
100 IU-PG
300 IU-PG
600 IU-PG
800 IU-PG
F1-2
F3-5
rh-hCG
rh-ahCG
rh-bhCG
Treatment

Figure 4.

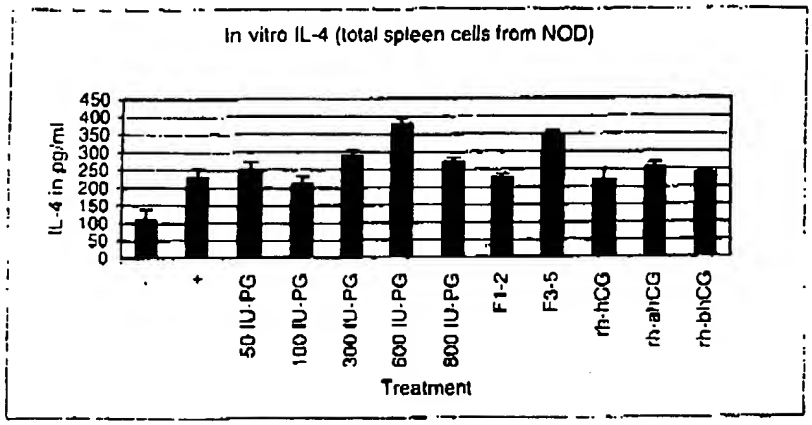

In vitro IL-4 (total spleen cells from NOD)
IL-4 in pg/ml
450
400
350
300
250
200
150
100
50
0
-
+
50 IU-PG
100 IU-PG
300 IU-PG
600 IU-PG
800 IU-PG
F1-2
F3-5
rh-hCG
rh-alhCG
rh-blhCG
Treatment

Figure 5.

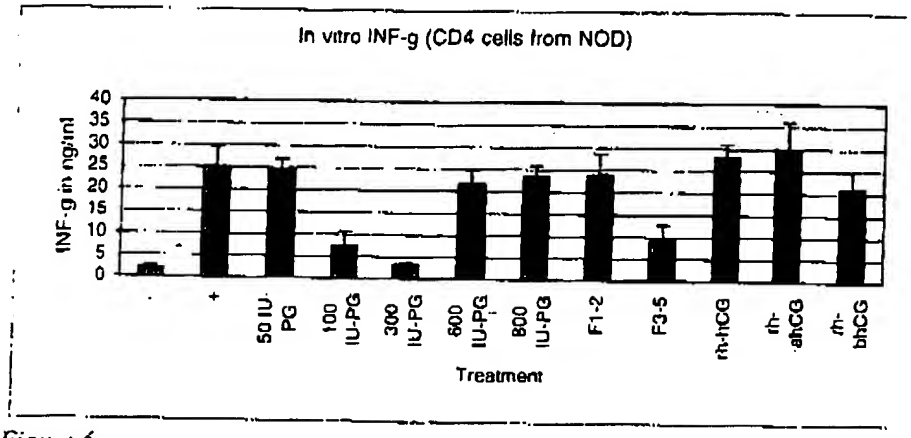

In vitro INF-g (CD4 cells from NOD)
INF-g in ng/ml
40
35
30
25
20
15
10
5
0
+
50 IU PG
100 IU-PG
300 IU-PG
600 IU-PG
800 IU-PG
F1-2
F3-5
rh-hCG
rh-ahCG
rh-bhCG
Treatment

Figure 6.

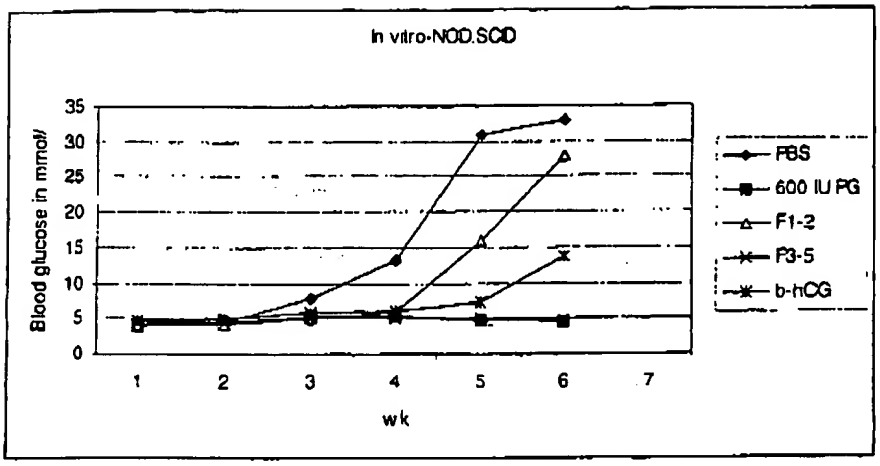

In vitro-NOD.SCID
Blood glucose in mmol/
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
wk
PBS
600 IU PG
F1-2
F3-5
b-hCG

Figure 7

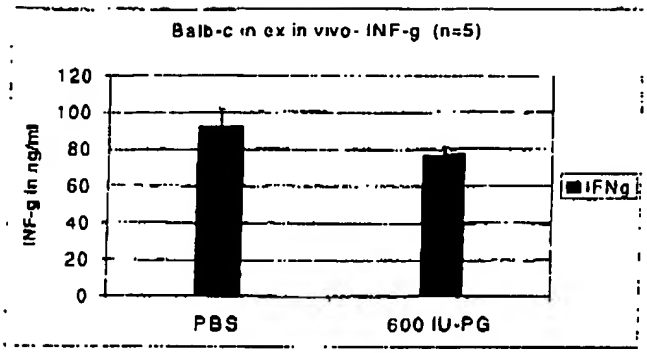


Figure 8.

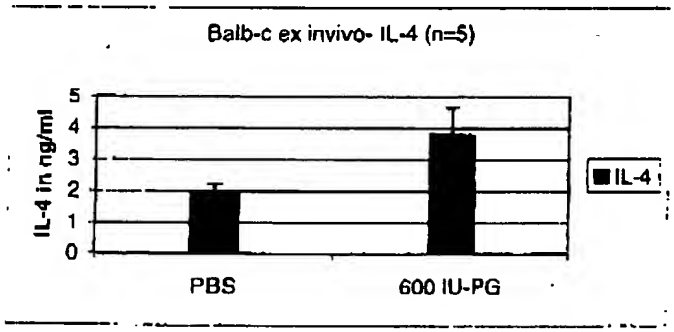


Figure 9.

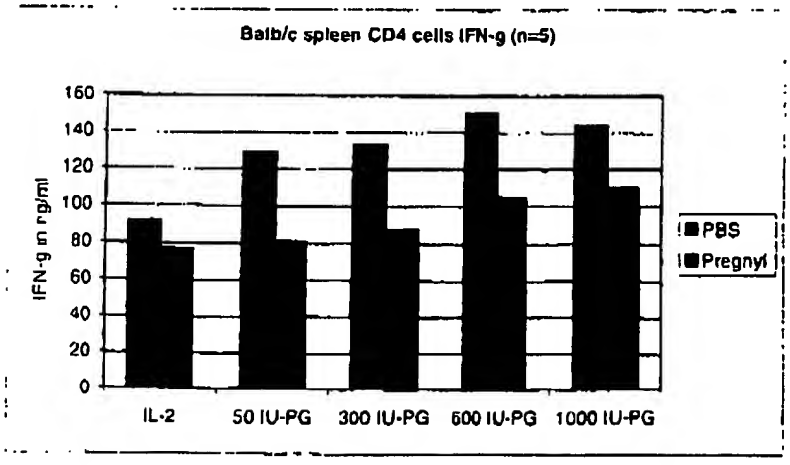


Figure 10.

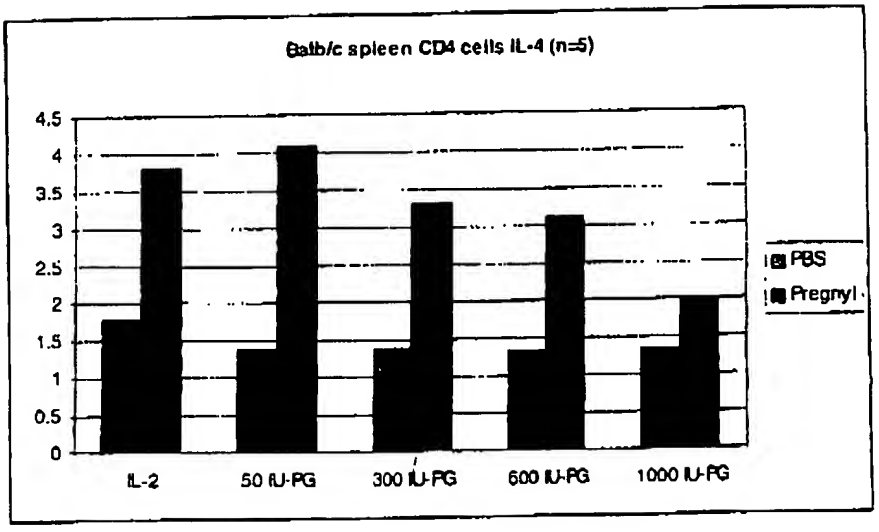

Balb/c spleen CD4 cells IL-4 (n=5)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
IL-2
50 IU-PG
300 IU-PG
600 IU-PG
1000 IU-PG
PBS
Pregnyl

Figure 11.

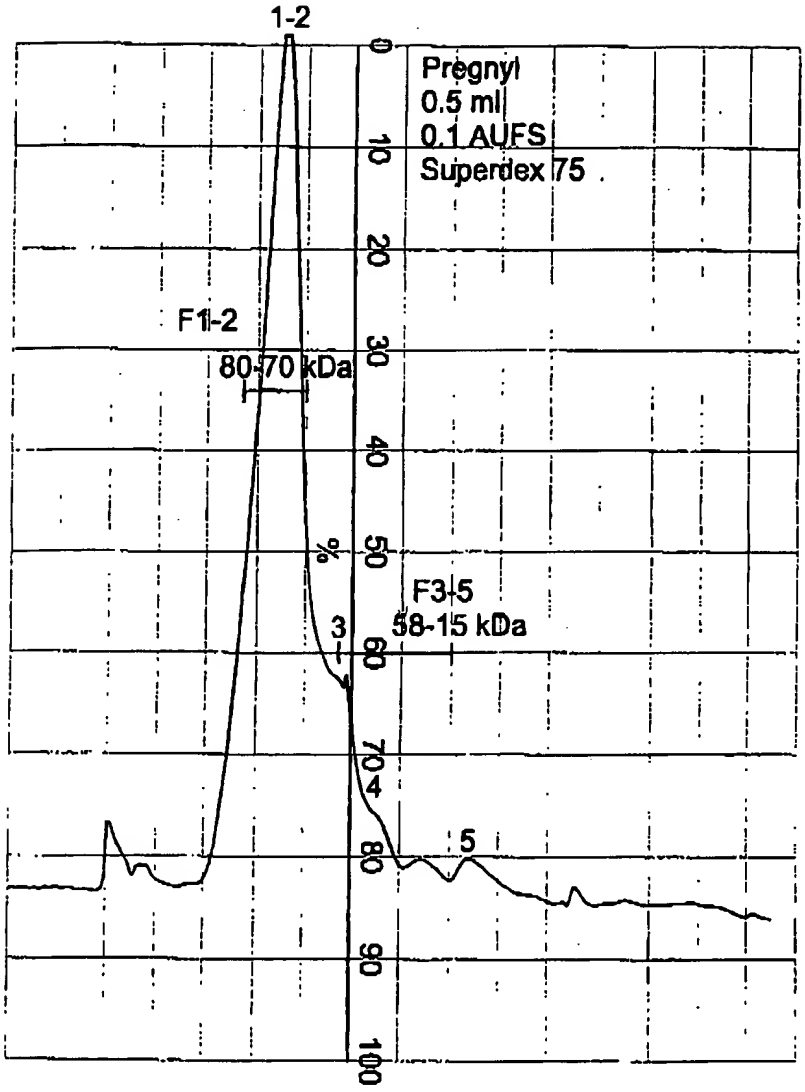

1-2
Pregnyl
0.5 ml
0.1 AUFS
Superdex 75
F1-2
80-70 kDa
%
F3-5
58-15 kDa
3
5
0
10
20
30
40
50
60
70
80
90
100

Figure 12

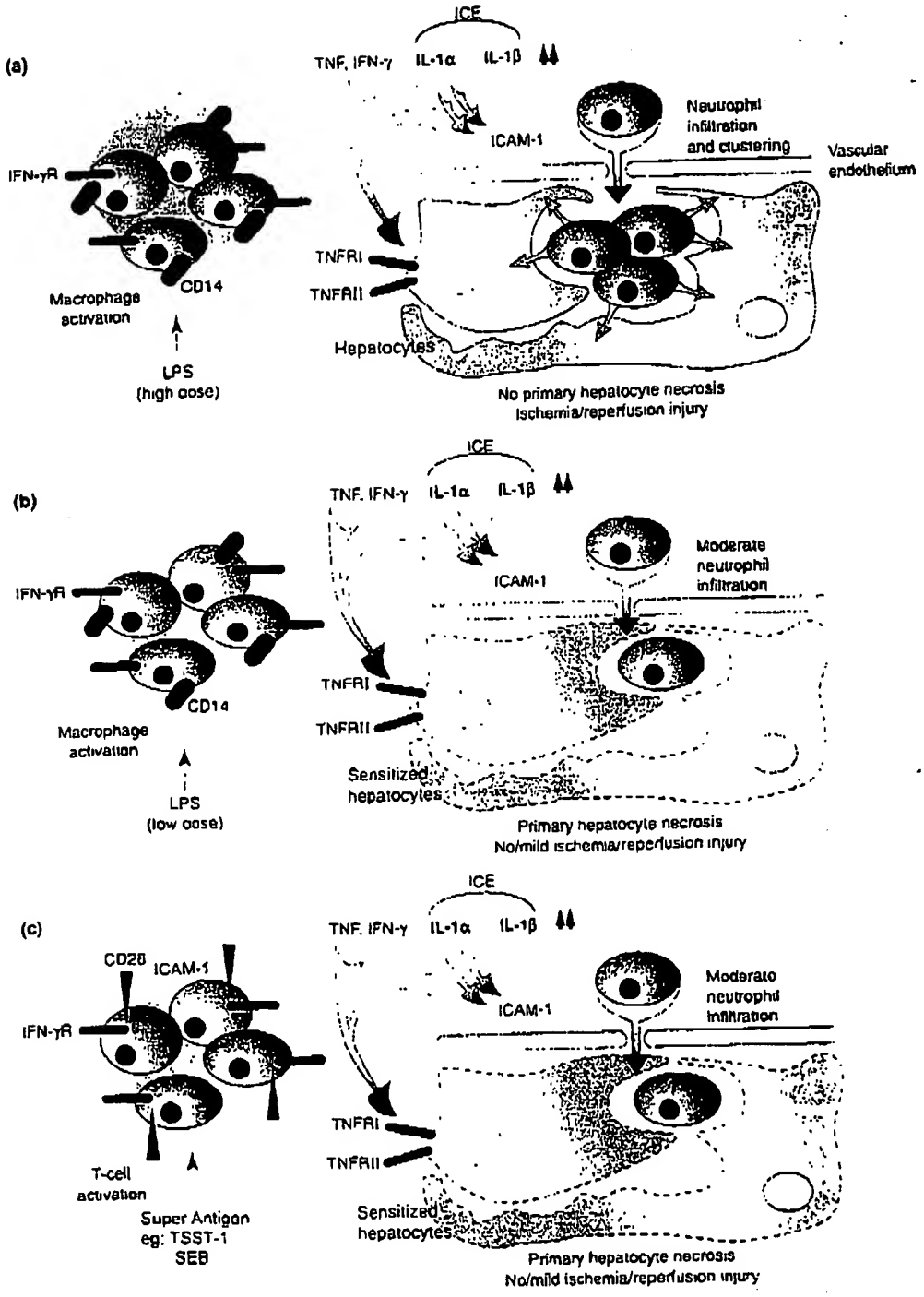

(a)
ICE
TNF, IFN-γ
IL-1α
IL-1β
ICAM-1
Neutrophil infiltration and clustering
Vascular endothelium
IFN-γR
CD14
Macrophage activation
LPS (high dose)
TNFRI
TNFRII
Hepatocytes
No primary hepatocyte necrosis Ischemia/reperfusion injury
(b)
ICE
TNF, IFN-γ
IL-1α
IL-1β
ICAM-1
Moderate neutrophil infiltration
IFN-γR
CD14
Macrophage activation
LPS (low dose)
TNFRI
TNFRII
Sensitized hepatocytes
Primary hepatocyte necrosis No/mild ischemia/reperfusion injury
(c)
ICE
TNF, IFN-γ
IL-1α
IL-1β
CD28
ICAM-1
ICAM-1
Moderate neutrophil infiltration
IFN-γR
T-cell activation
Super Antigen eg: TSST-1 SEB
TNFRI
TNFRII
Sensitized hepatocytes
Primary hepatocyte necrosis No/mild ischemia/reperfusion injury

Figure 13

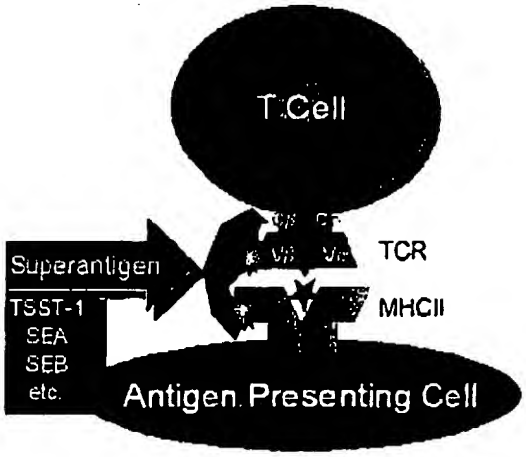

T Cell
TCR
MHCII
Superantigen
TSST-1
SEA
SEB
etc.
Antigen Presenting Cell

Figure 14

Figure 15

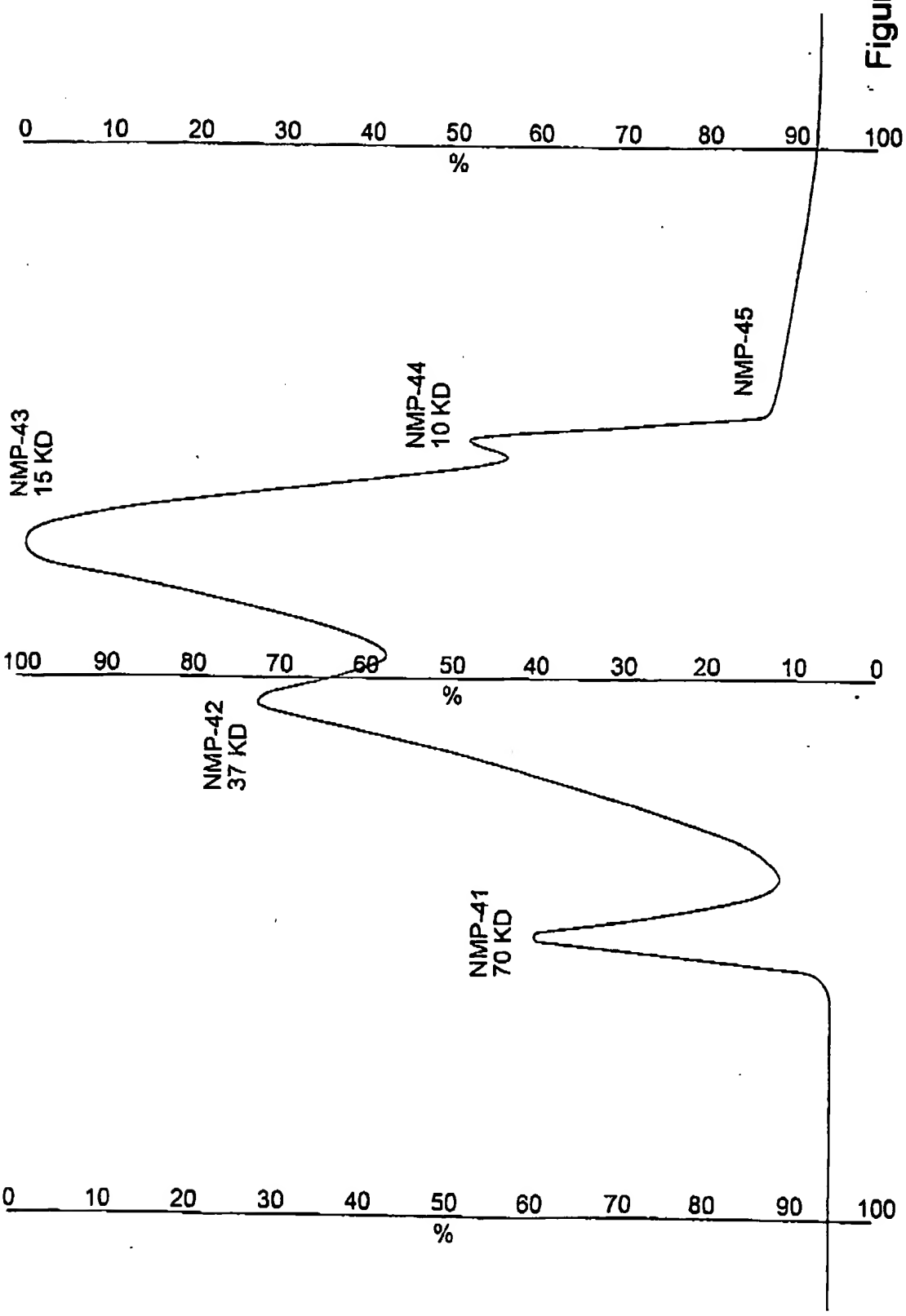

0 10 20 30 40 50 60 70 80 90 100
%
NMP-45
NMP-44
10 KD
NMP-43
15 KD
100 90 80 70 60 50 40 30 20 10 0
%
NMP-42
37 KD
NMP-41
70 KD
0 10 20 30 40 50 60 70 80 90 100
%

Figure 16

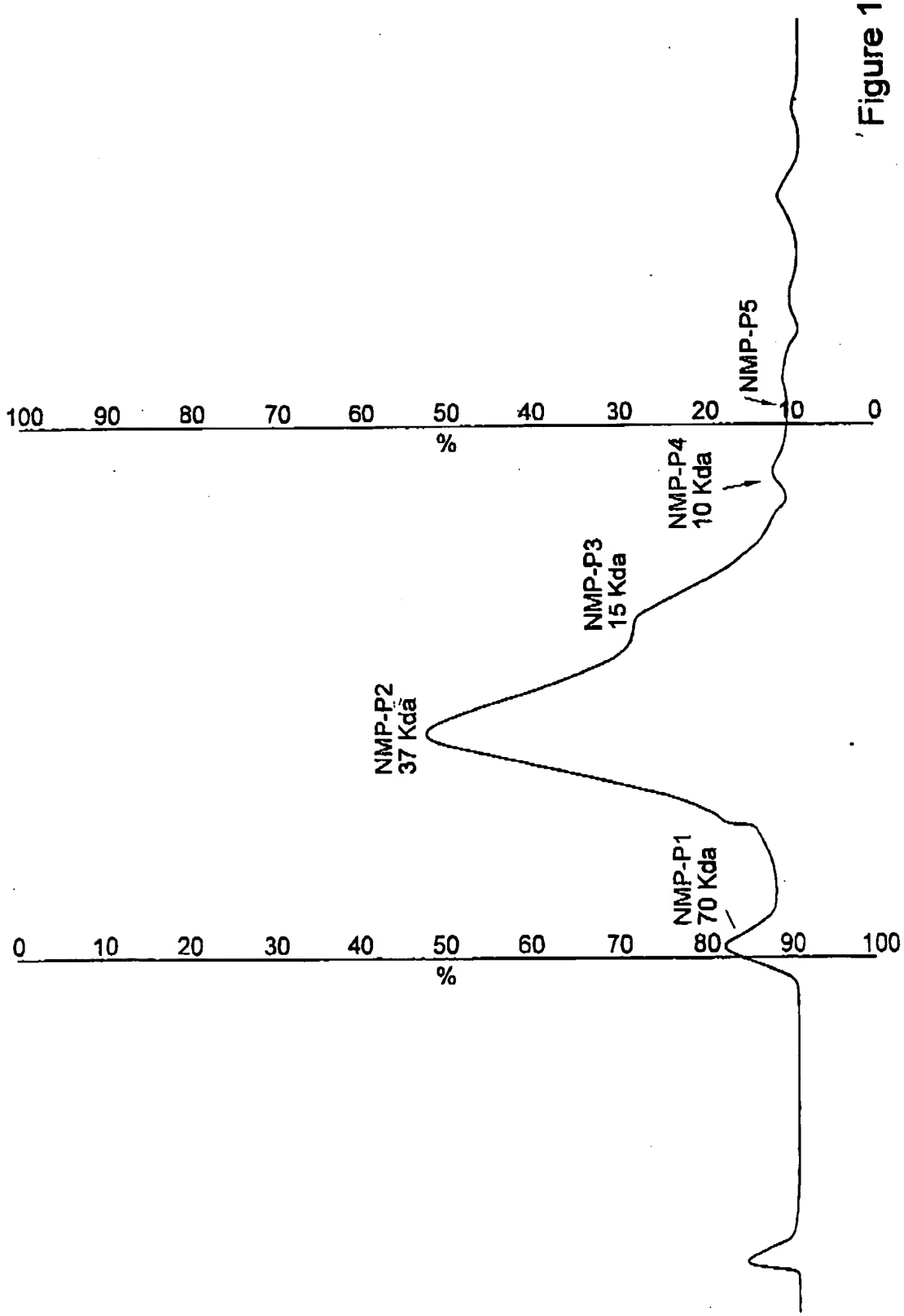


Figure 17

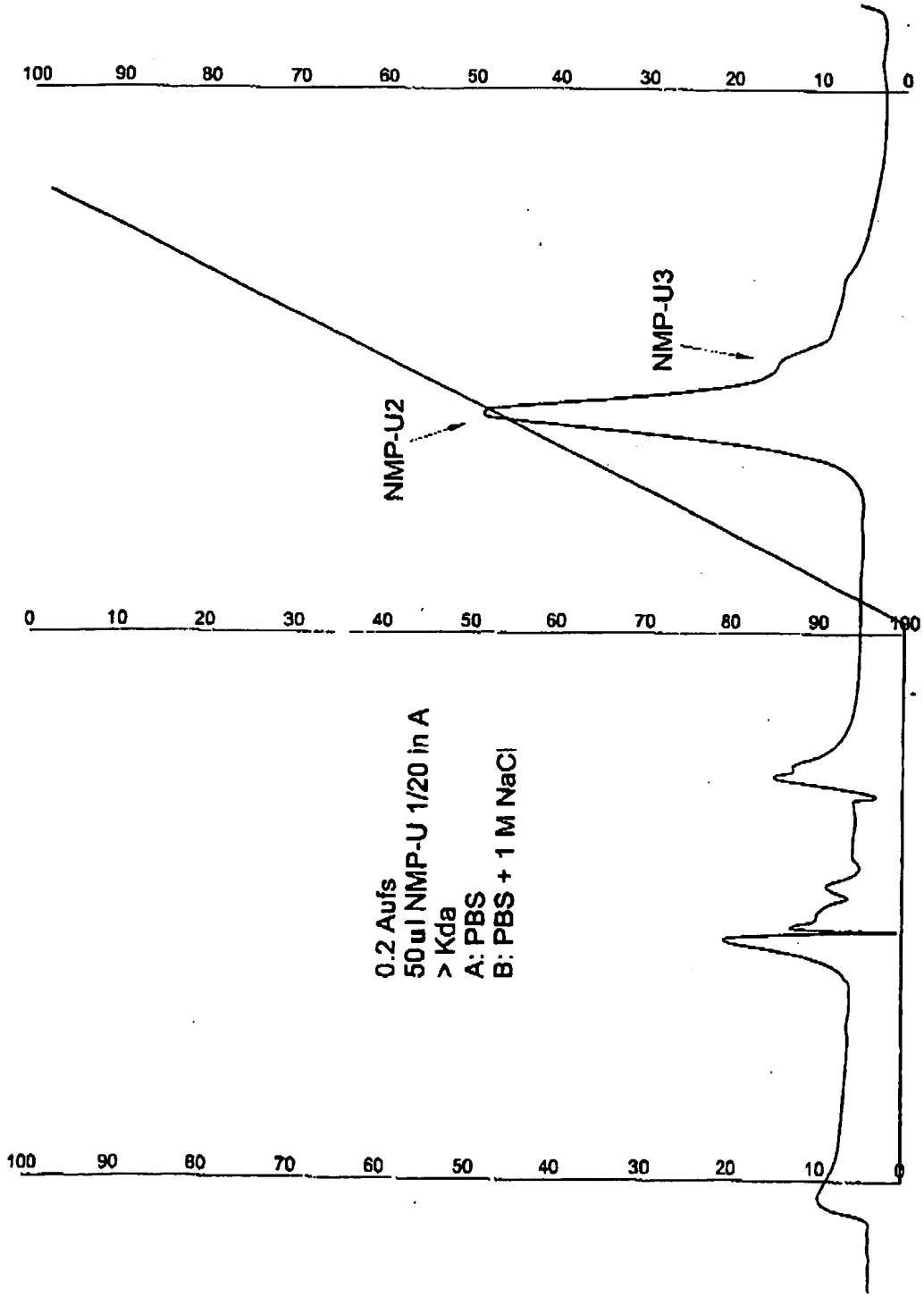

100 90 80 70 60 50 40 30 20 10 0
NMP-U3
NMP-U2
0 10 20 30 40 50 60 70 80 90 100
0.2 Aufs
50 ul NMP-U 1/20 in A
> Kda
A: PBS
B: PBS + 1 M NaCl
100 90 80 70 60 50 40 30 20 10 0

Figure 18

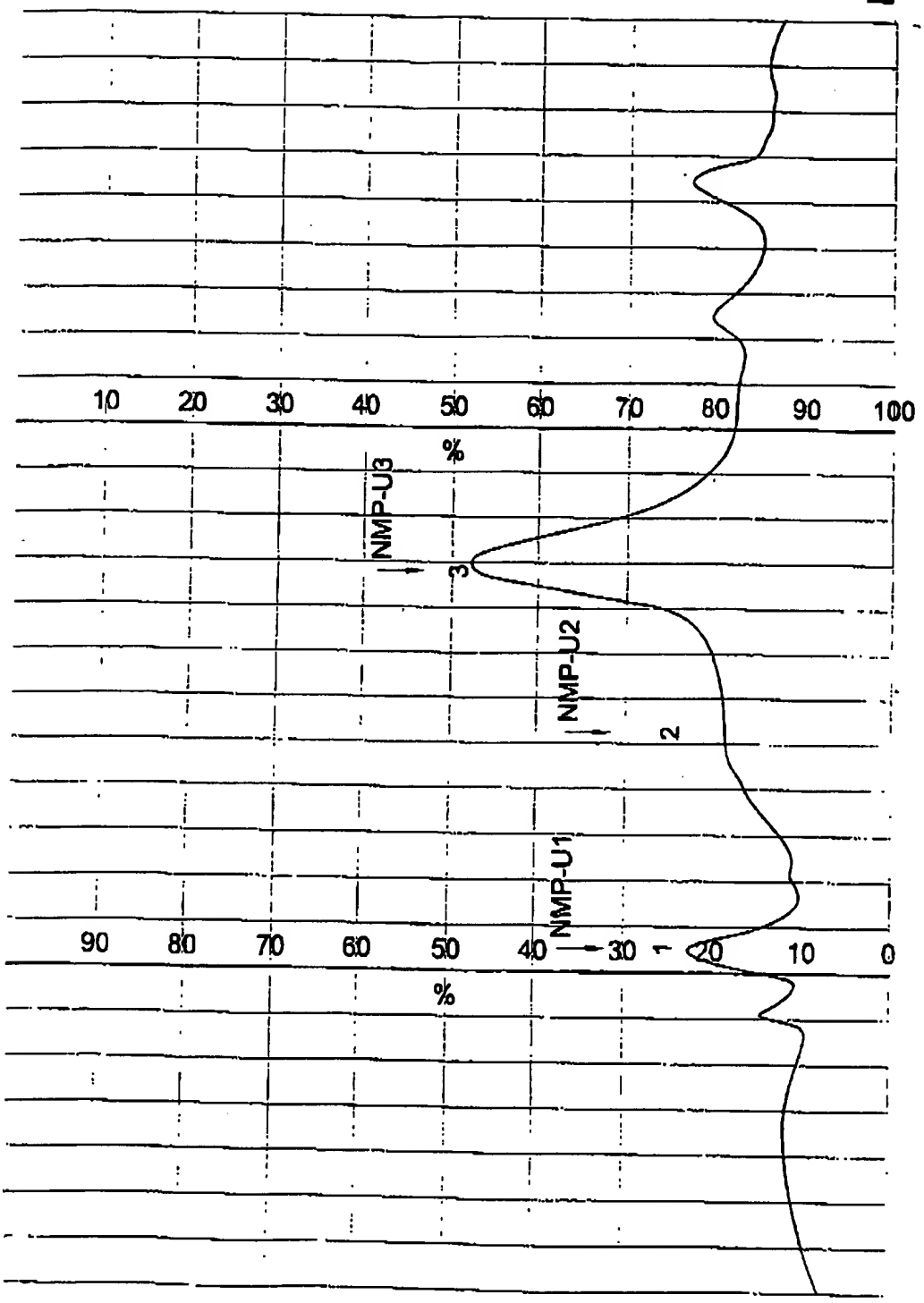
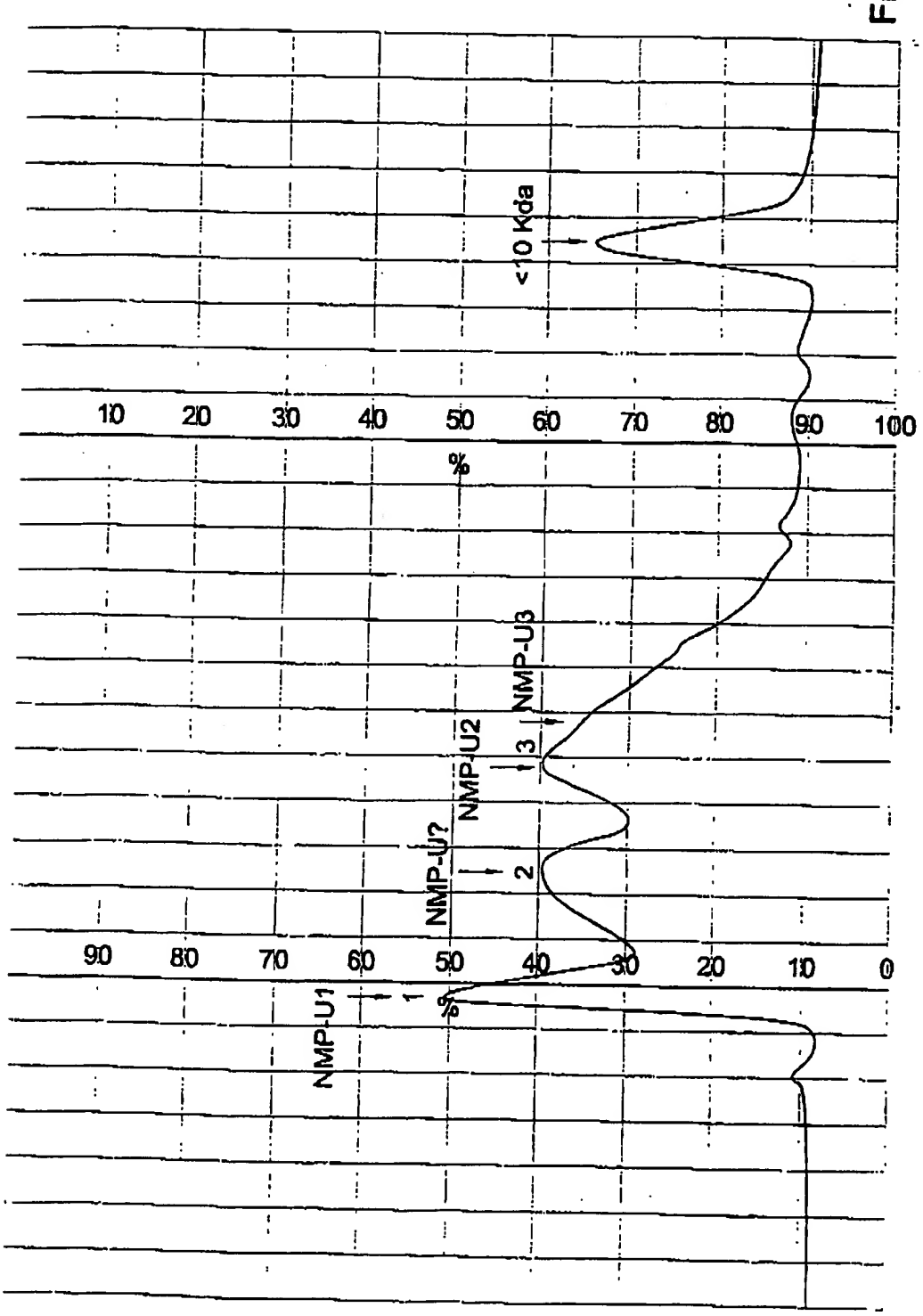

10 20 30 40 50 60 70 80 90 100
%
NMP-U3
3
NMP-U2
2
NMP-U1
90 80 70 60 50 40 30 1 20 10 0
%

Figure 19


<10 Kda
10 20 30 40 50 60 70 80 90 100
%
NMP-U3
3
NMP-U2
NMP-U?
2
90 80 70 60 50 40 30 20 10 0
%
NMP-U1
1

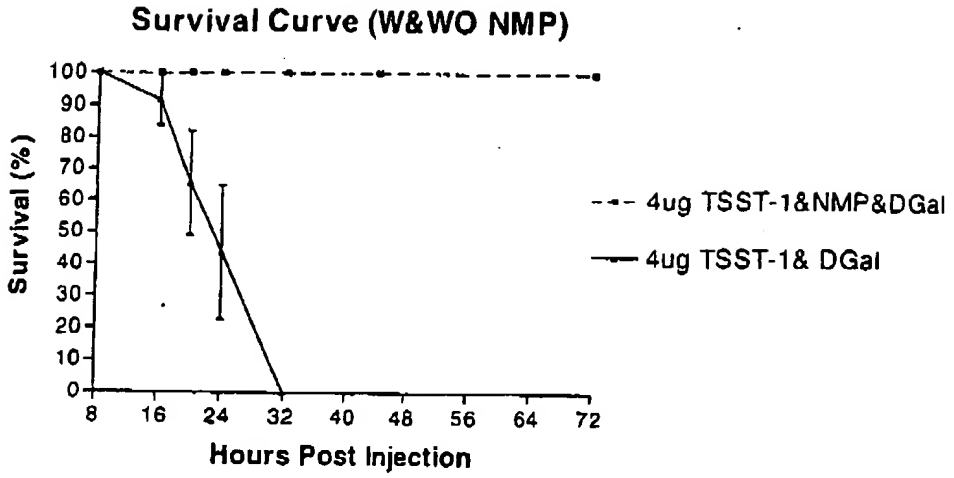

Survival Curve (W&WO NMP)
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
8
16
24
32
40
48
56
64
72
Hours Post Injection
4ug TSST-1&NMP&DGal
4ug TSST-1& DGal

Figure 20

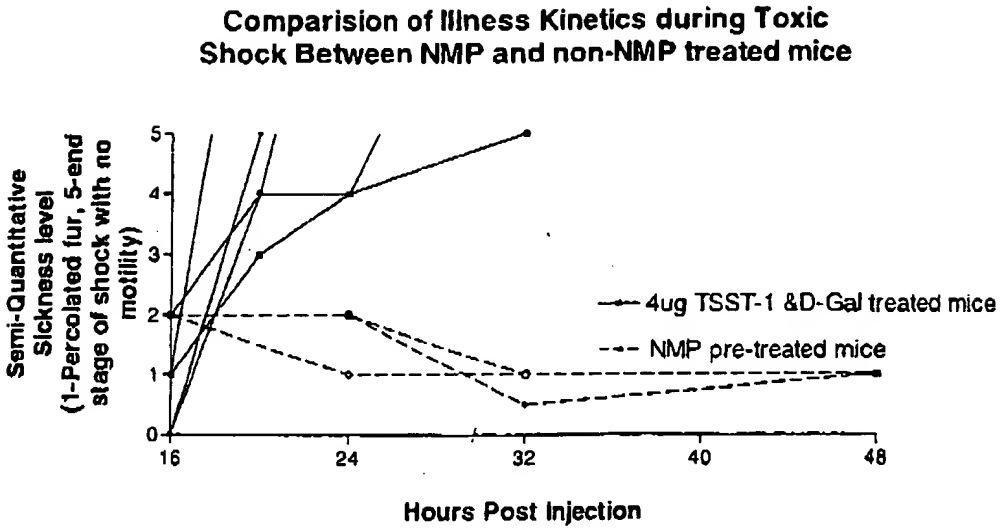

Comparision of Illness Kinetics during Toxic Shock Between NMP and non-NMP treated mice
Semi-Quantitative Sickness level (1-Percolated fur, 5-end stage of shock with no motility)
0
1
2
3
4
5
16
24
32
40
48
Hours Post Injection
4ug TSST-1 &D-Gal treated mice
NMP pre-treated mice

Figure 21

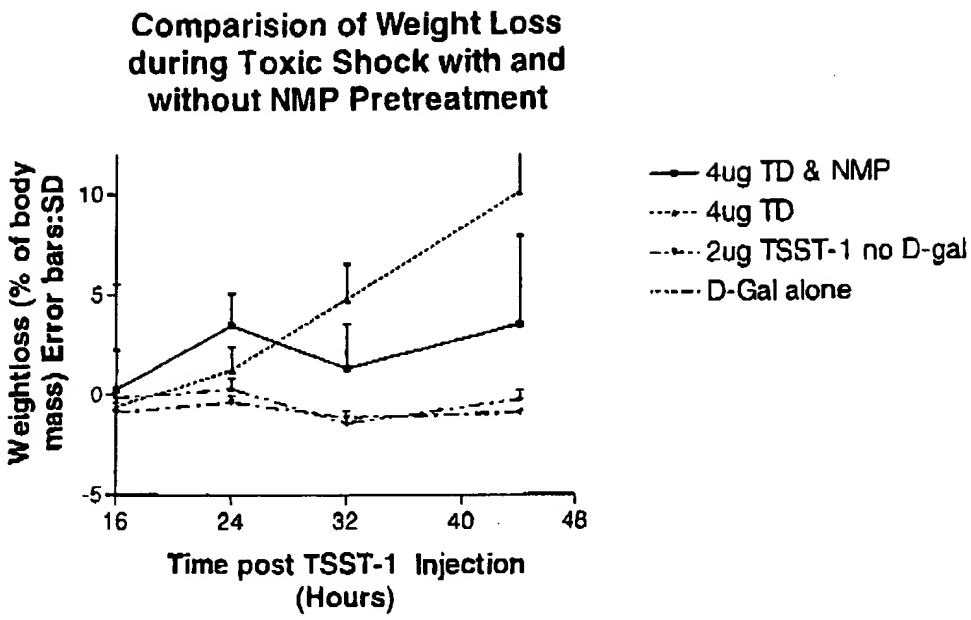

Comparision of Weight Loss during Toxic Shock with and without NMP Pretreatment
Weightloss (% of body mass) Error bars:SD
10
5
0
-5
16
24
32
40
48
Time post TSST-1 Injection (Hours)
4ug TD & NMP
4ug TD
2ug TSST-1 no D-gal
D-Gal alone

Figure 22

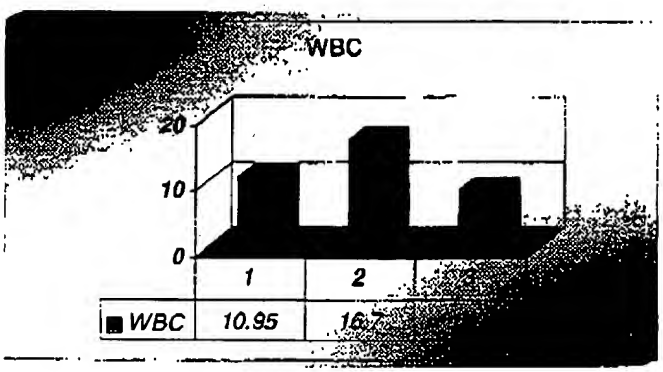


Figure 23

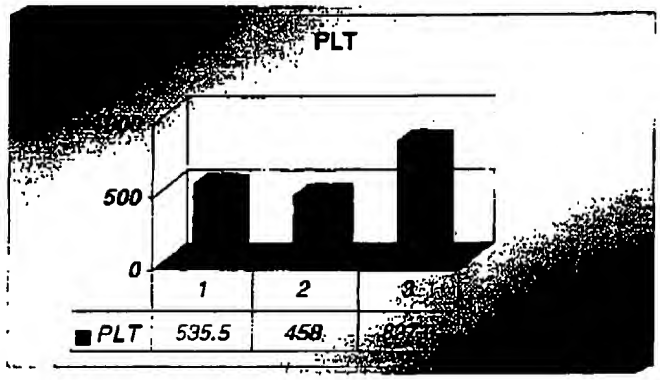


Figure 24

Figure 25

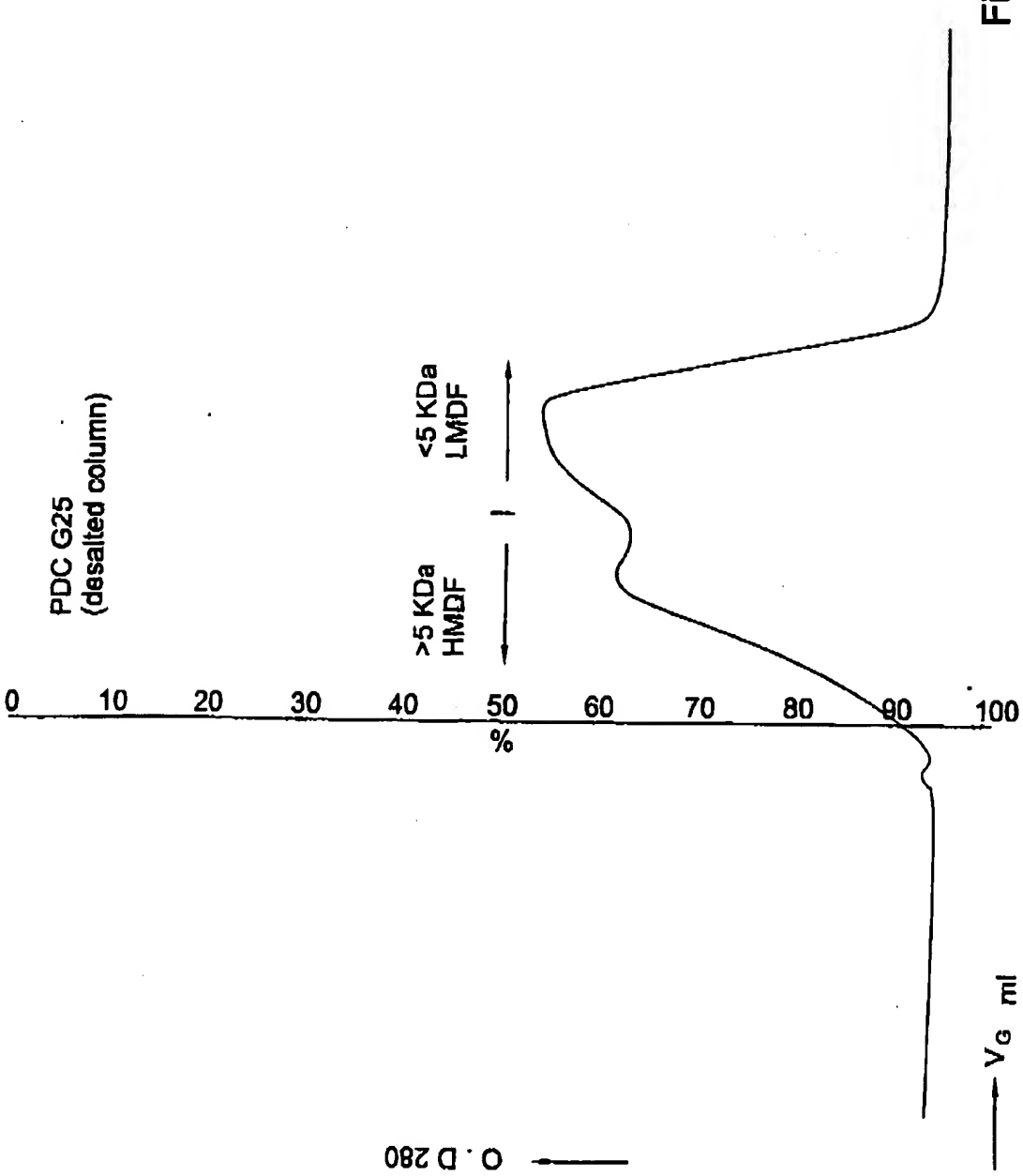
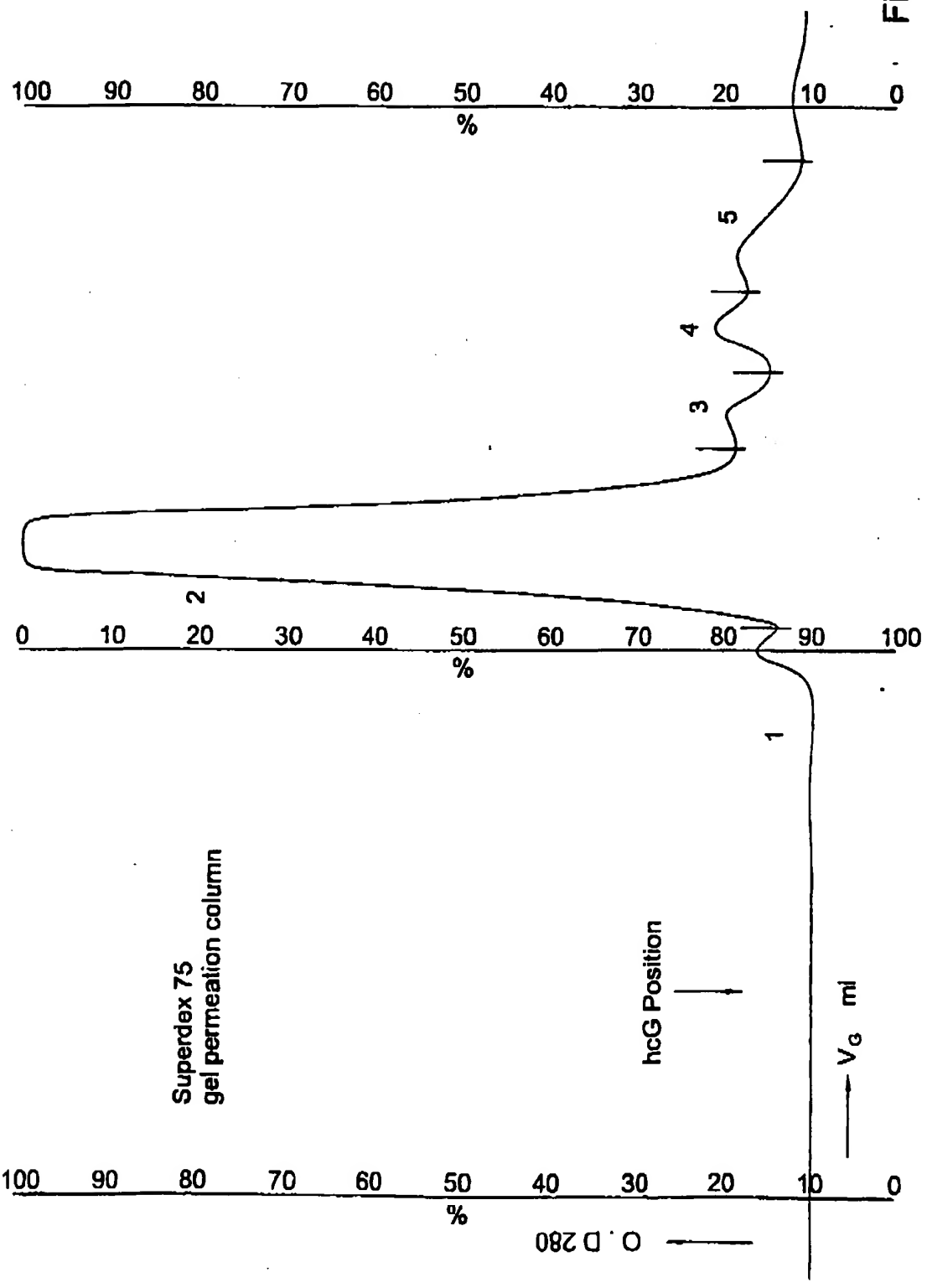

PDC G25
(desalted column)
<5 KDa
LMDF
>5 KDa
HMDF
0
10
20
30
40
50
60
70
80
90
100
%
O . D 280
V_G ml

Figure 26


100 90 80 70 60 50 40 30 20 10 0
%
5
4
3
2
0 10 20 30 40 50 60 70 80 90 100
%
1
Superdex 75
gel permeation column
hcG Position
V_G ml
100 90 80 70 60 50 40 30 20 10 0
%
O . D 280

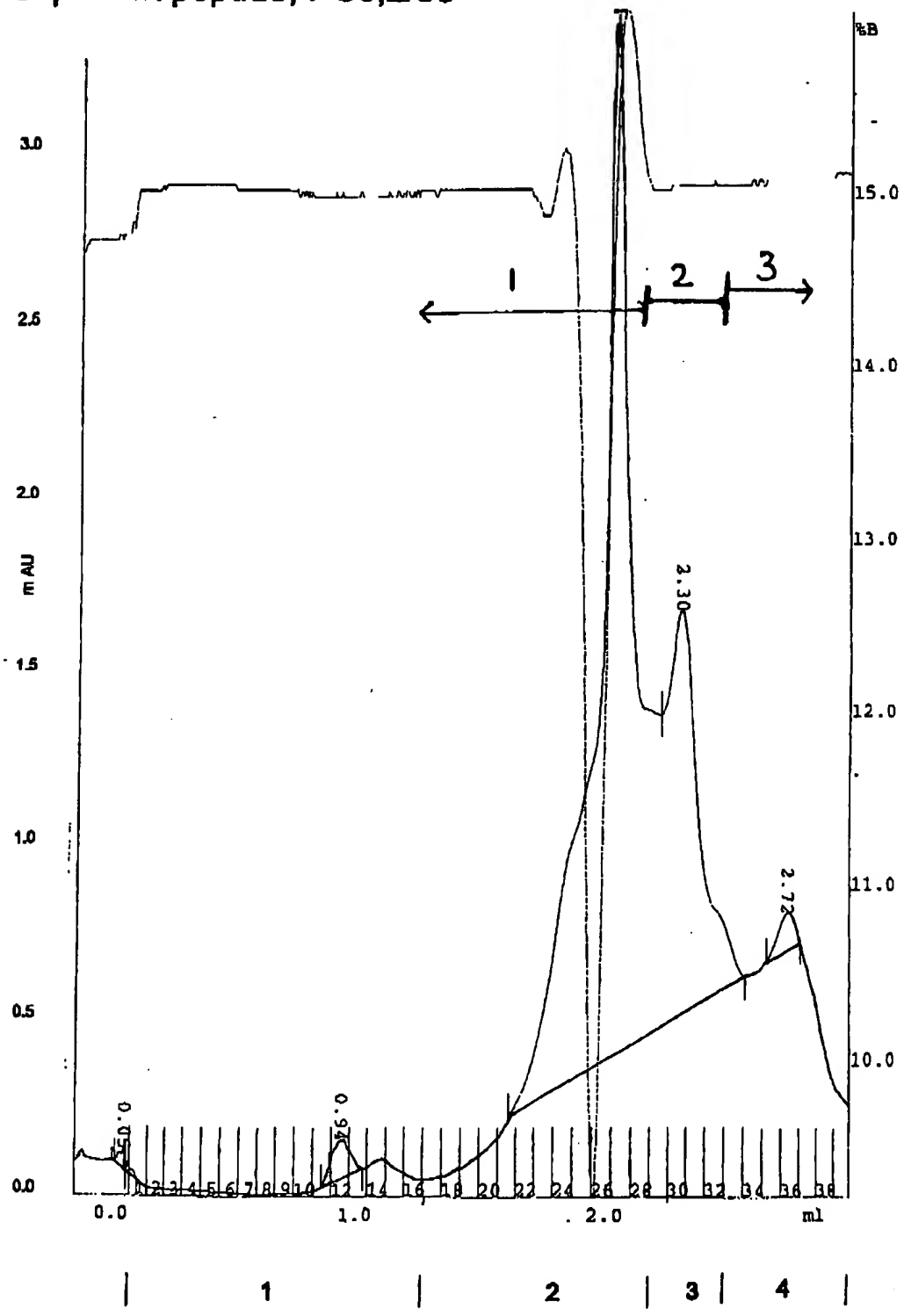

superdex peptide, PC3,2/30
%B
mAU
3.0
2.5
2.0
1.5
1.0
0.5
0.0
15.0
14.0
13.0
12.0
11.0
10.0
1
2
3
2.30
2.72
0.05
0.96
0.0
1.0
2.0
ml
1
2
3
4

Figure 27

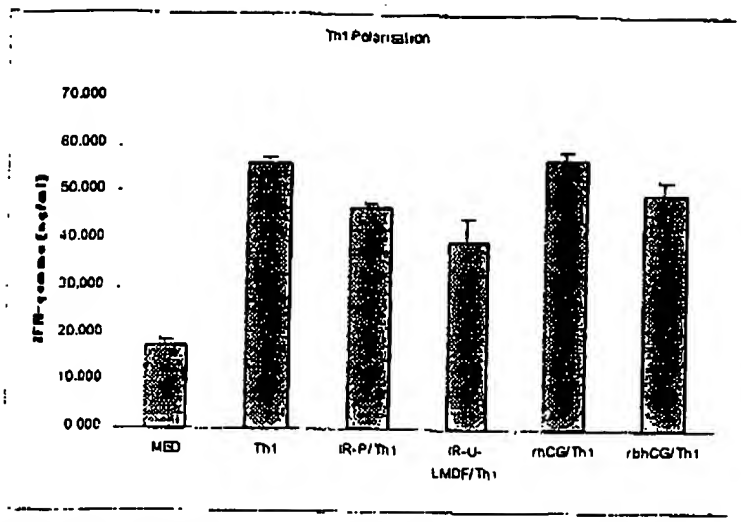


Figure 28. This figure shows that there is strong inhibition of IFN-gamma production found with IR-P and IR-U/LMDF on CD4+ cells polarizing towards Th1 phenotype (in vivo). There was only a moderate inhibition of IFN-gamma production observed with recombinant beta-hCG and no effect was seen with recombinant hCG as compare to control (MED).

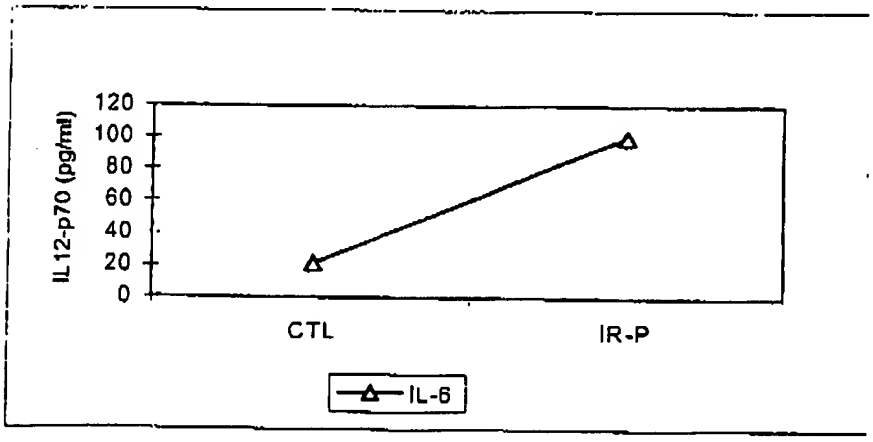
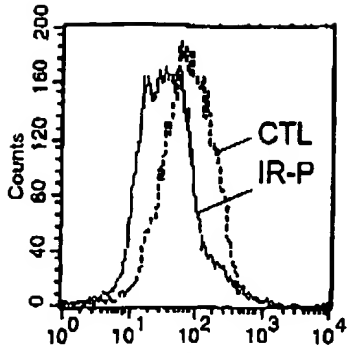
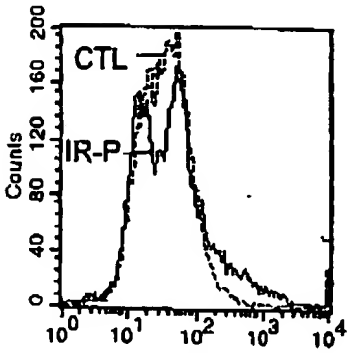
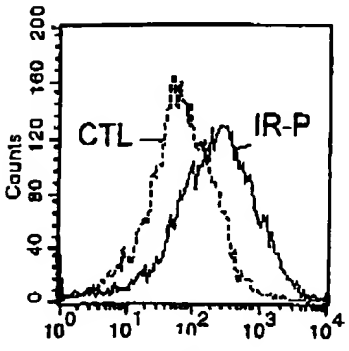


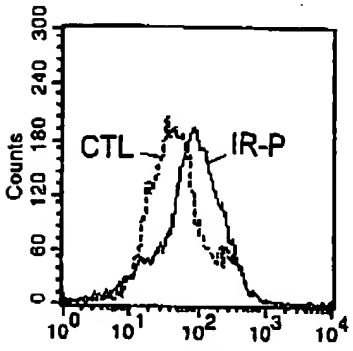
figure 45

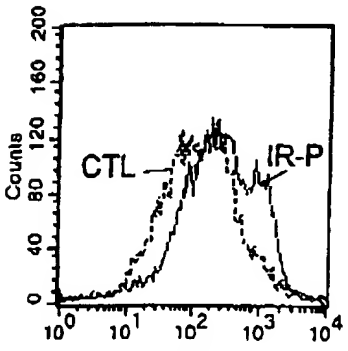
Figure 29

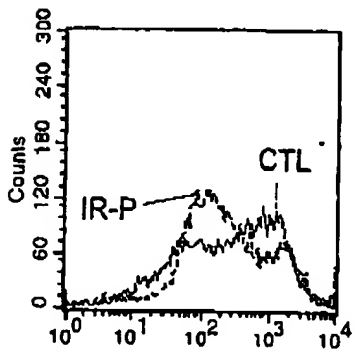

Counts
CTL
IR-P
CD1d

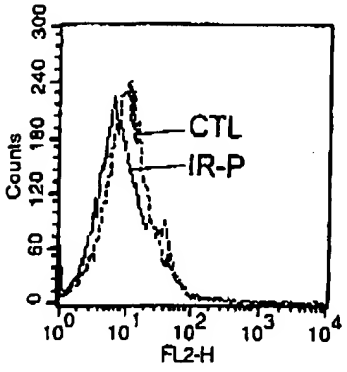

Counts
CTL
IR-P
CD14


Counts
CTL
IR-P
CD31


Counts
CTL
IR-P
CD43


Counts
CTL
IR-P
CD95


Counts
CTL
IR-P
ER-MP20/LY6C


Counts
CTL
IR-P
FL2-H
NEG. CONTR.

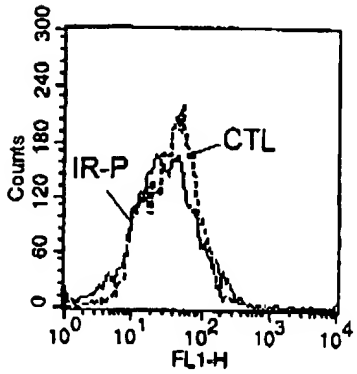
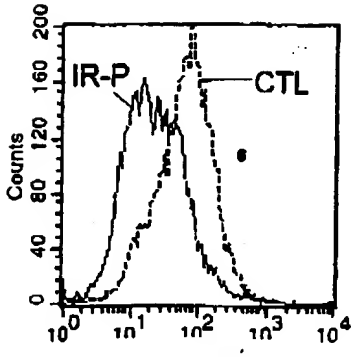
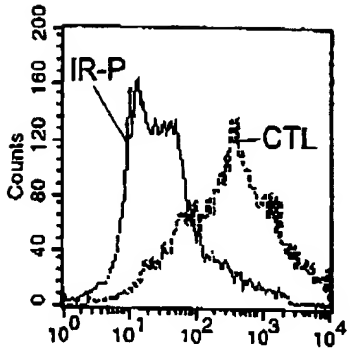
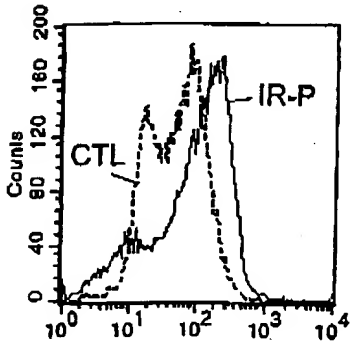
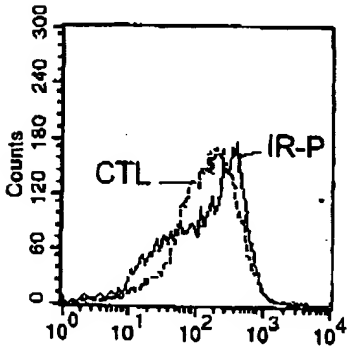

Counts
CTL
IR-P
FL1-H
NEG. CONTR.

Figure 29


IR-P
CTL
Counts
ER-MP58


IR-P
CTL
Counts
F4/80


CTL
IR-P
Counts
E-cad


CTL
IR-P
Counts
class I

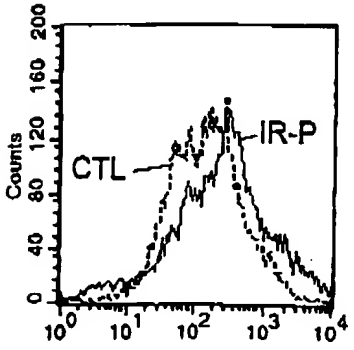
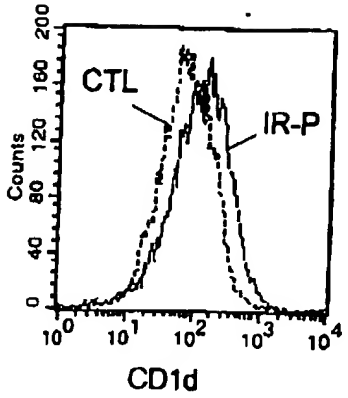
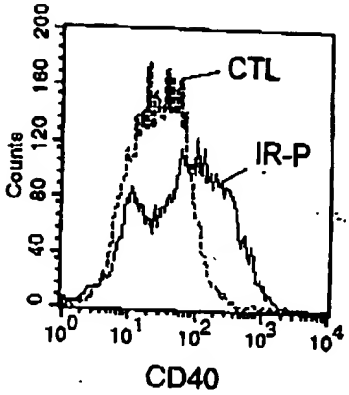
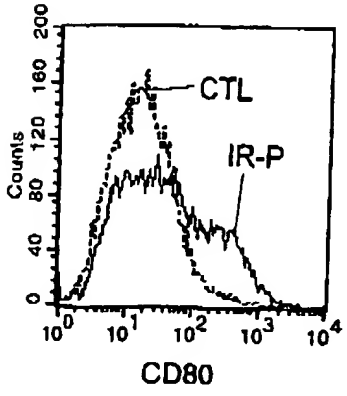
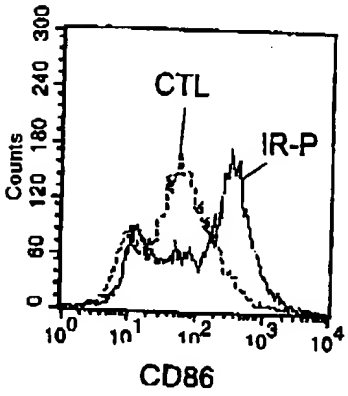

CTL
IR-P
Counts
class II

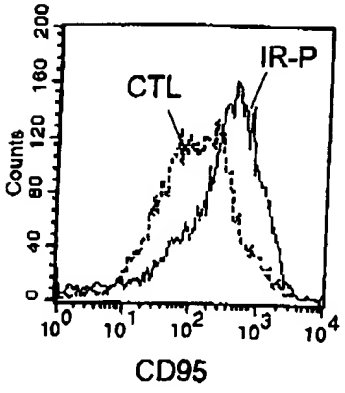
Figure 30

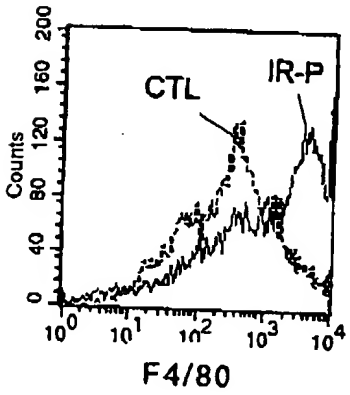

CTL
IR-P
Counts
CD1d

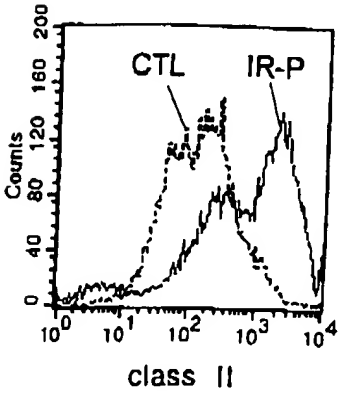

CTL
IR-P
Counts
CD40


CTL
IR-P
Counts
CD80


CTL
IR-P
Counts
CD86


CTL
IR-P
Counts
CD95


CTL
IR-P
Counts
F4/80


CTL
IR-P
Counts
class II

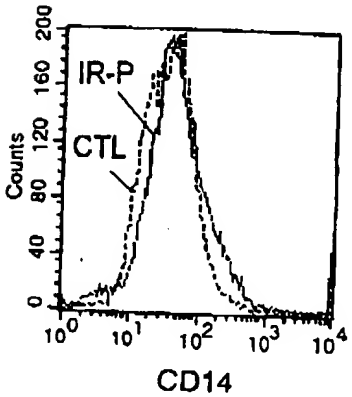

IR-P
CTL
Counts
CD14

Figure 30

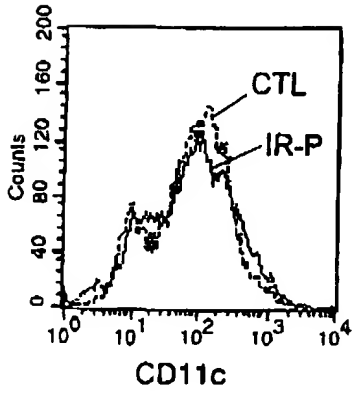
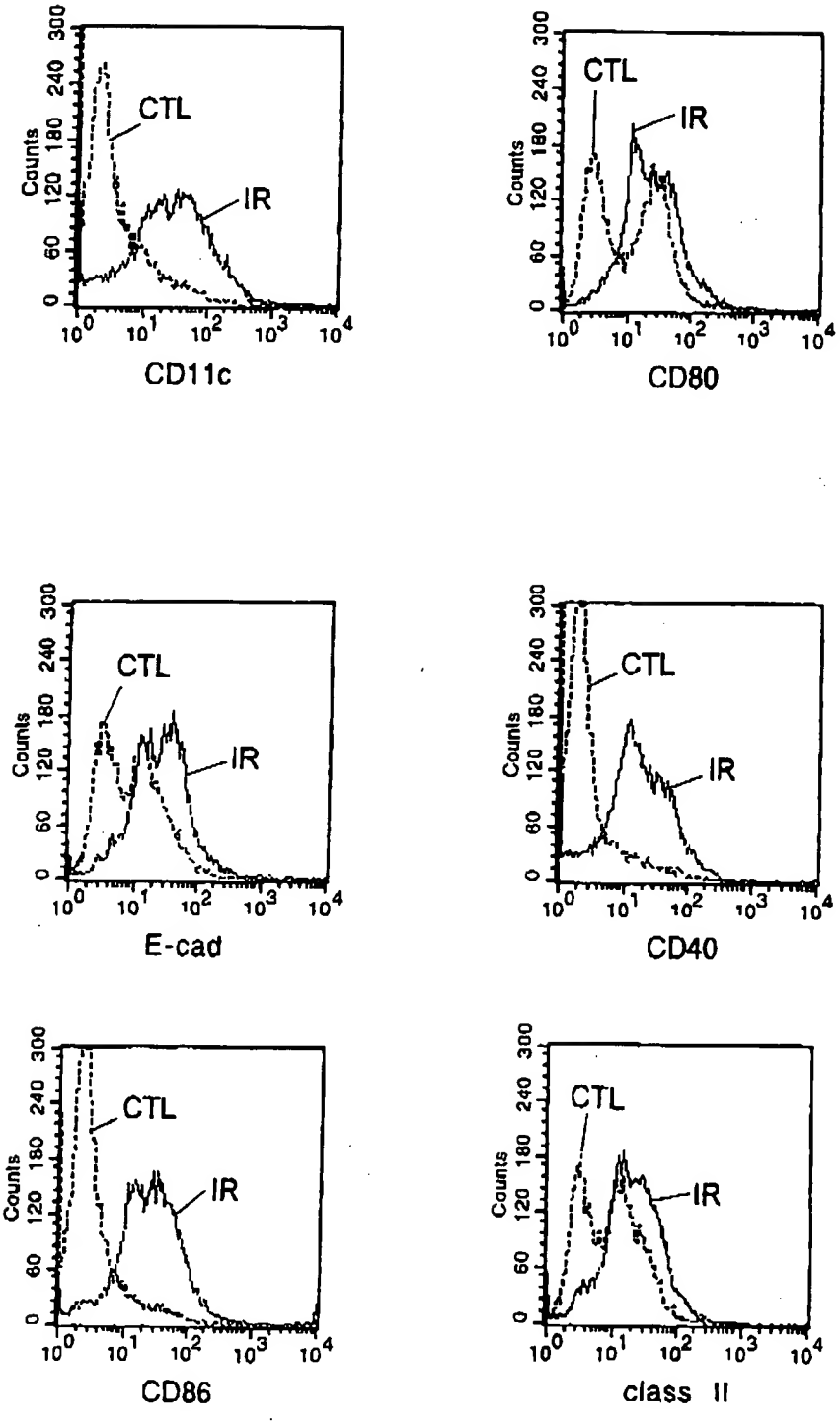


Figure 31



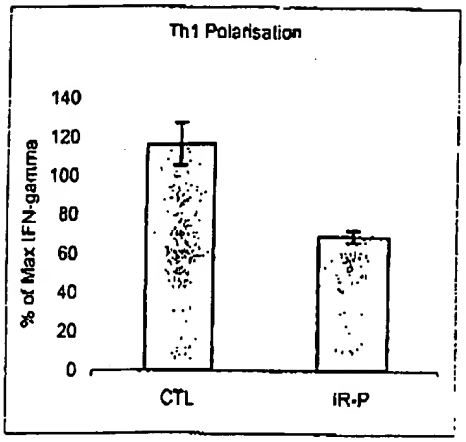


Figure 32 shows that due to the IR-P treatment in Balb/c mice the CD4+ cell are shifted towards Th2 phenotype, showed by the inhibition of IFN-gamma production as compare to control (CTL) group.

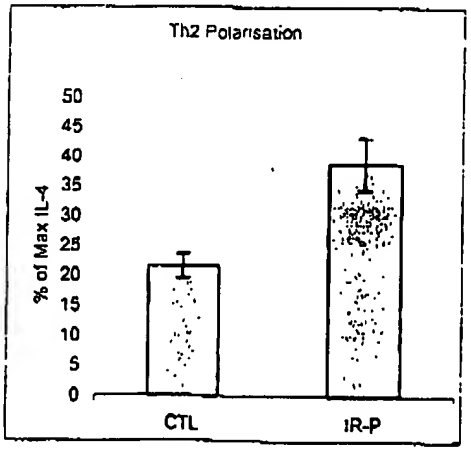


Figure 34 shows that due to the IR-P treatment in Balb/c mice the CD4+ cell are shifted towards Th2 phenotype, showed by the increase in IL-4 production as compare to control (CTL) mice.

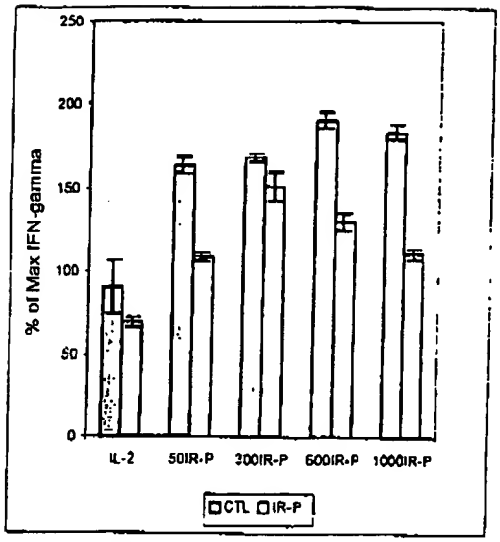


Figure 36 shows that CD4+ T cells from PBS and IR-P mice treated (in vivo) with different doses of IR-P (in vitro) show increase in IFN-gamma production which suggest the shift towards Th1 phenotype (see also figure 37).

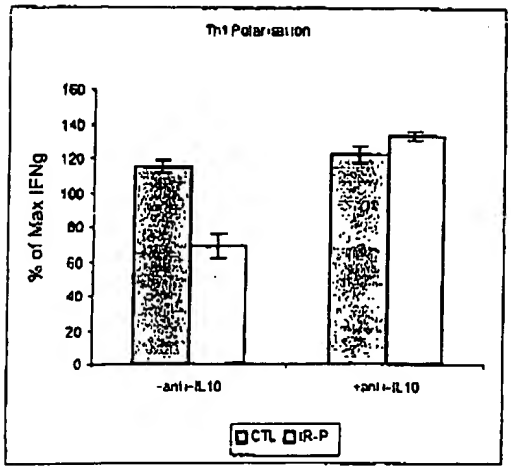


Figure 38 shows an increase in IFN-gamma production in Th1 polarization conditions in IR-P group, which suggests that the promoting effect of IR-P on Th2 subset is at least IL-10 dependent (for detail see text).

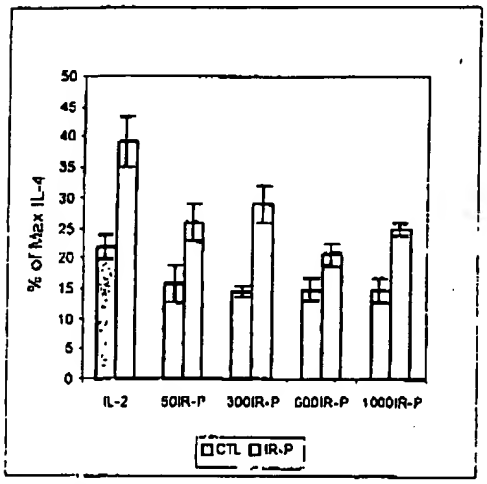


figure 37 shows that CD4+ T cells from PBS and IR-P mice treated (in vivo) with different doses of IR-P (in vitro) show decrease in IL-4 production which suggest the shift towards Th1 phenotype (see also figure 36).

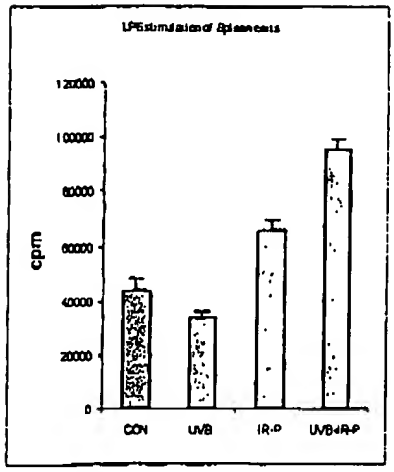


Figure 46.

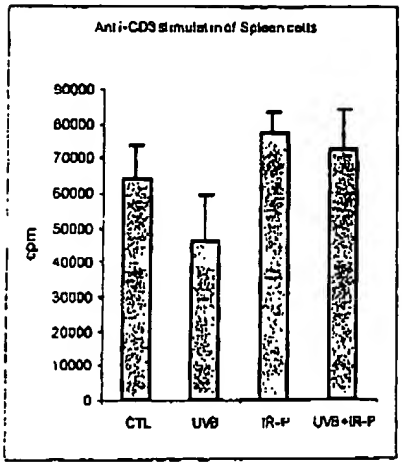


Figure 47.

LPS and anti-CD3 stimulated proliferation of spleen cells from UVB and IR treated Balb/c mice. Reduction in LPS and anti-CD3 proliferation was observed in UVB treated Balb/c mice (figure 46, 47)) while IR or combined IR and UVB-irradiated treated mice had increase LPS and anti-CD3 stimulated proliferation (figure 46, 47).

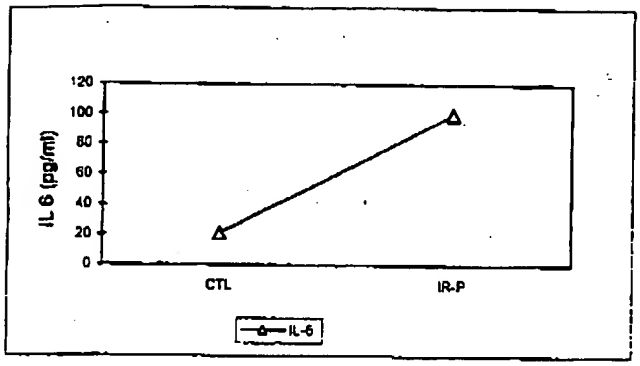


figure 45 shows that LPS stimulated spleens cells from IR treated Balb/c mice produce high level of IL-6 (ex vivo) as compare to control (CTL) group treated with PBS

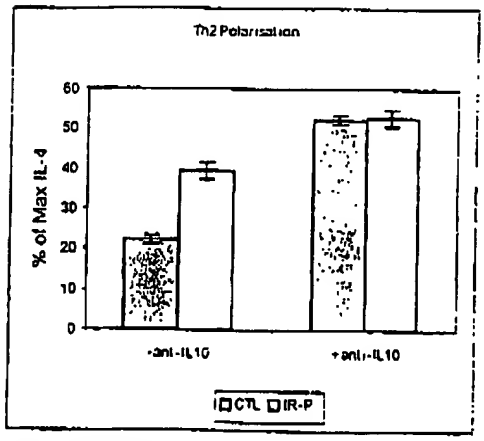


Figure 39 shows increase in IL-4 production in Th2 polarization conditions seen with anti-IL10 invitro treatment in control (CTL) group and in IR-P group. This suggests involvement of IL-10 in Th1/Th2 polarisation (for detail see text).

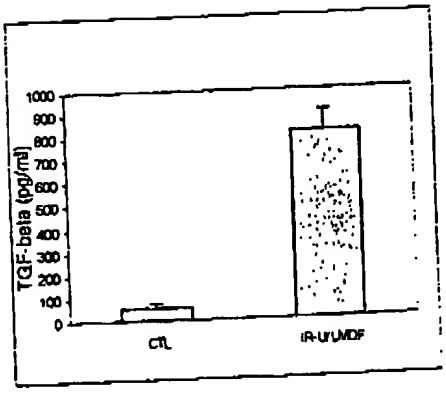


Figure 43

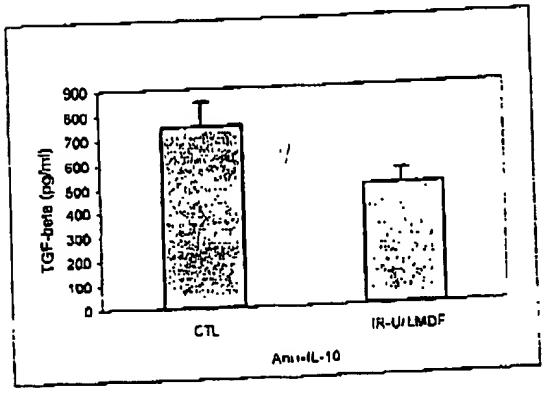


Figure 44 A.

FOR DETAIL SEE DOCUMENT

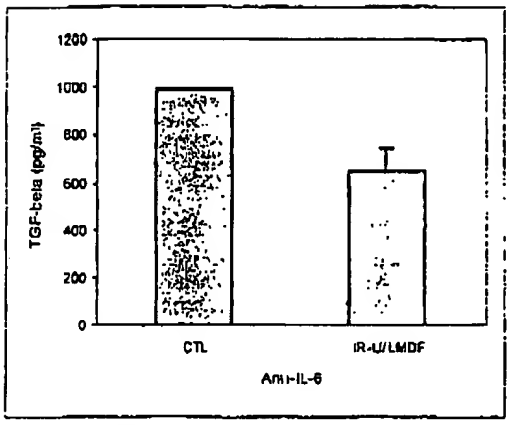


Figure 44 B

FOR DETAIL SEE DOCUMENT

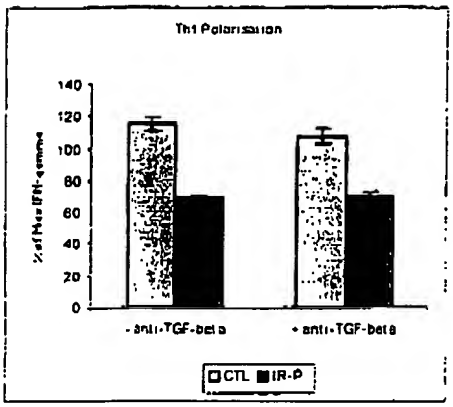


Figure 40.

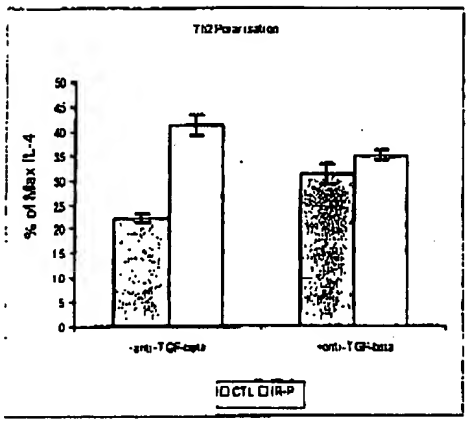


Figure 41

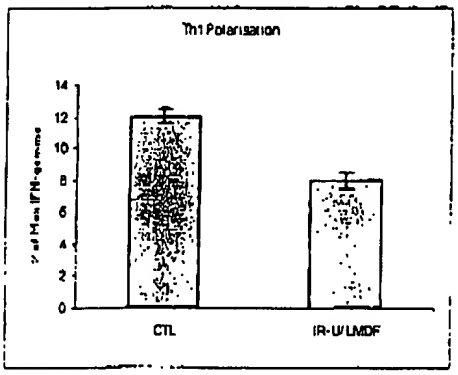


Figure 33.

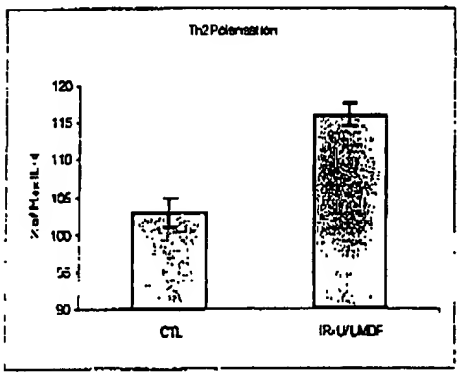


Figure 35.

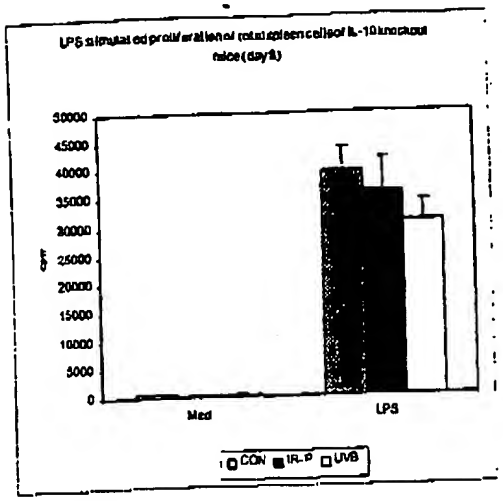


Figure 50

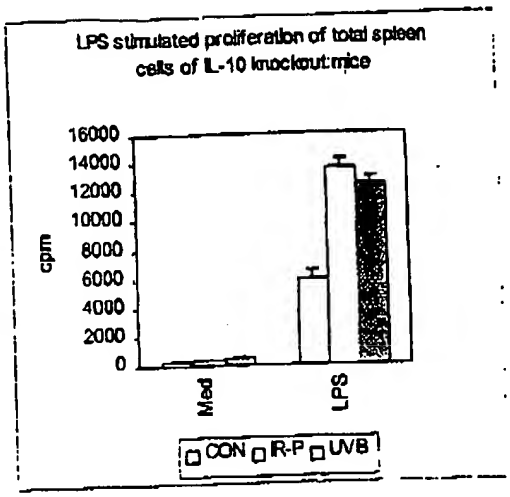


Figure 51

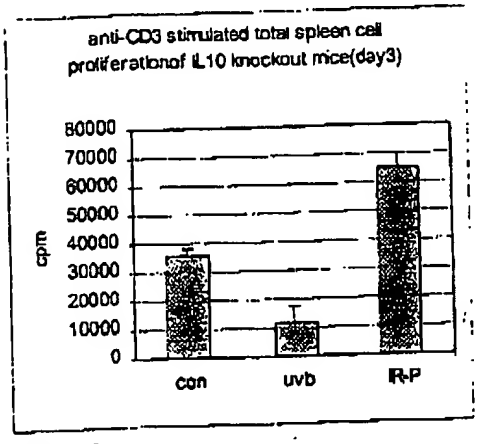


Figure 48

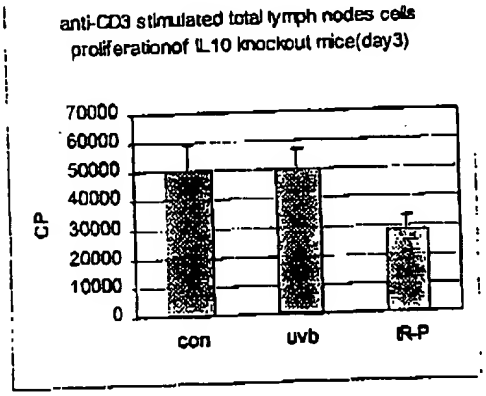


Figure 49

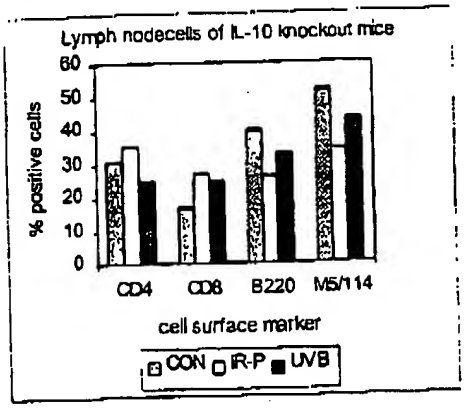


Figure 52

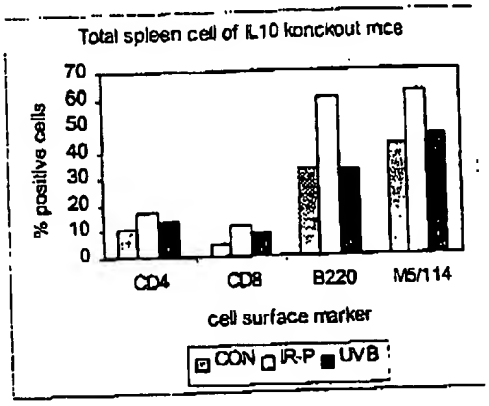


Figure 53

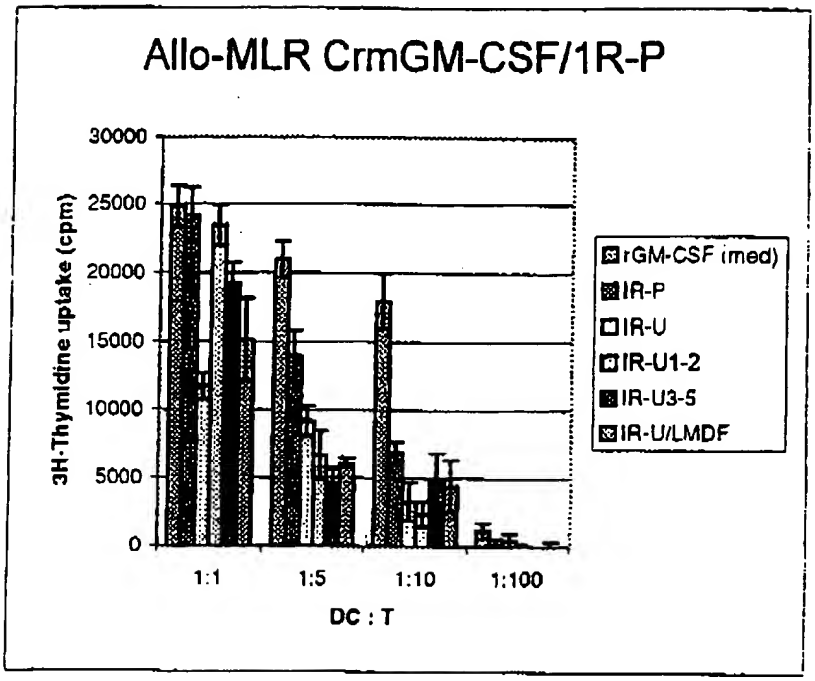
Mab	Med	IR-P	IR-U	IR-U3-5	IR-U/LMDF
CD1d	4.9	3.2	2.4	2.8	2.8
CD14	0.0	0.6	2.7	1.0	0.8
CD40	0.0	0.0	0.0	0.0	0.0
CD80	0.3	0.0	0.0	0.0	0.0
CD86 (all)	1.9	0.8	0.1	0.5	0.6
CD95 (all)	5.3	4.1	12.8	5.6	5.6
CD95L	0.2	0.3	0.2	0.0	0.0
ER-MP58	3.9	2.6	1.7	0.0	1.1
F4/80 (all)	39.5	20.1	1.3	2.2	0.0
RB6.8C5		3.6	5.8	5.0	4.1
E-cad (all)	1.9	4.5	0.5	0.5	0.9
MHC II	13.8	7.8	9.3	6.3	0.0

Figure54

Mab	Med	IR-P	IR-U	IR-U3-5	IR-U/LMDF
CD1d	4.9	7.0	11.8	9.5	9.5
CD14	0.0	1.0	0.9	1.9	1.2
CD40	0.0	0.6	4.4	5.5	3.8
CD80	0.3	0.3	0.9	0.7	0.6
CD80 (fractic)			8.0 (37%)	16.0 (20%)	12.8 (20%)
CD86 (all)	1.9	3.3	19.7	10	11.5
CD95	5.3		15.2	16	16
ER-MP58	3.9	5.2	6.1	7.7	7.0
F4/80 (all)	39.5	32.2	108.8	136.9	155.
RB6.8C5		7.7	8.2	4.0	4.3
E-cad (all)	1.9	2.1	3.2	3.3	1.9
MHC II (all)	13.8	18.1	108.8	94.5	109.6

Figure 55

Figure 56



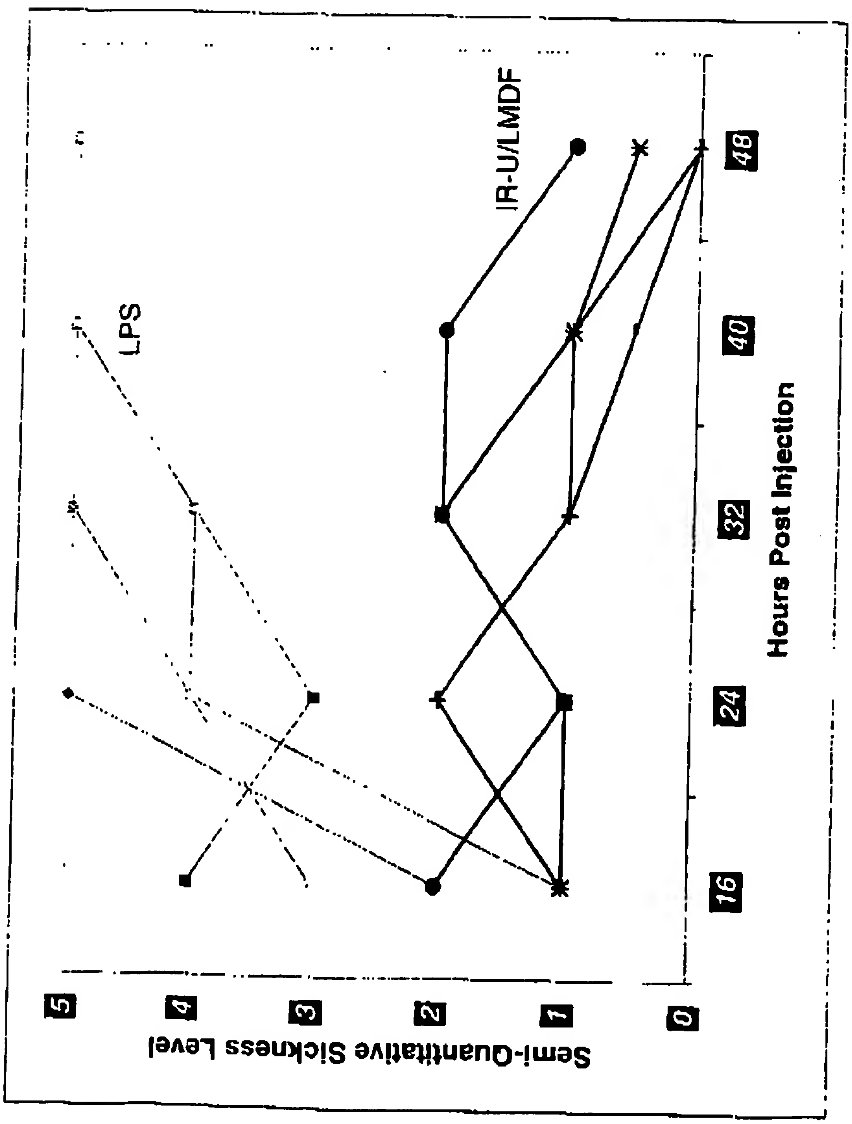


Figure 57

FOR DETAIL, SEE DOCUMENT

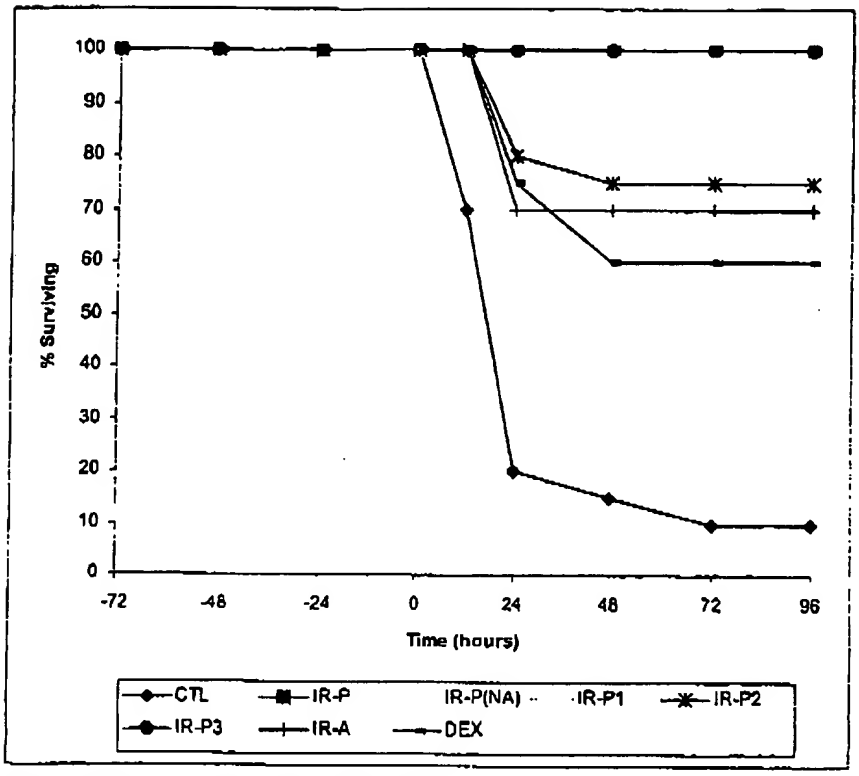


Figure 58.To determine the effect of high-dose LPS treatment in IR treated mice. Balb/c mice (n=30) were injected intraperitoneally with LPS (150 mg/kg) and survival was assessed daily 5 days. PBS-treated Balb/c mice succmbed to shock between days 1 and 2 after high-dose LPS injection, with only 10% of the animals were alive on day 5. In constrast, 100% of IR-P, or its fraction IR-P1, IR-P3 treated mice were alive on day 5 ($P<0.001$), while IR-P2, IR-A and Dexamethasone treated mice demonstrated around 70% of surviving .

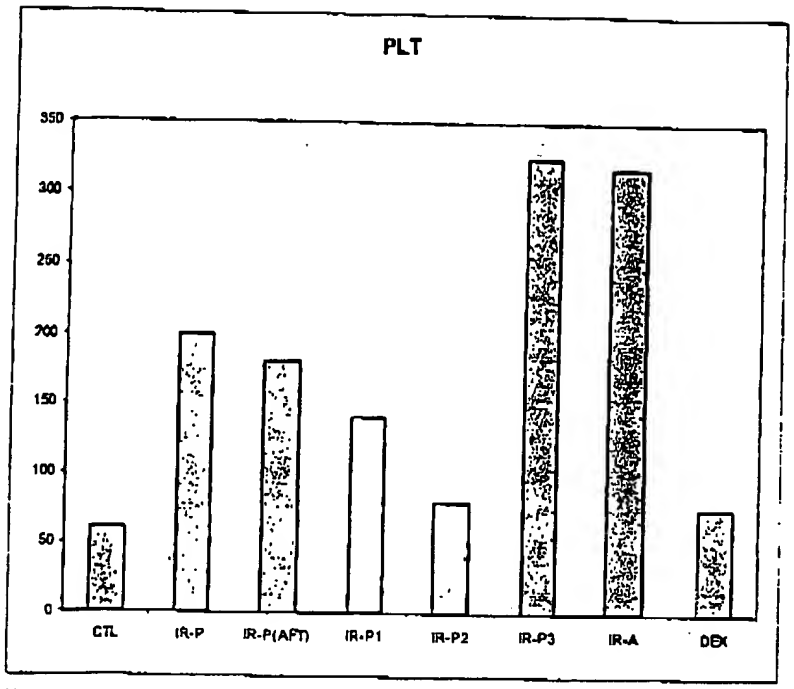


Figure 59 shows that IR-A, IR-P and its fraction IR-P1, IR-P3 have all platelets counts within normal range (100-300 x 10^9), while control, IR-P2 and Dexamethasone treated mice have platelets counts below normal range.

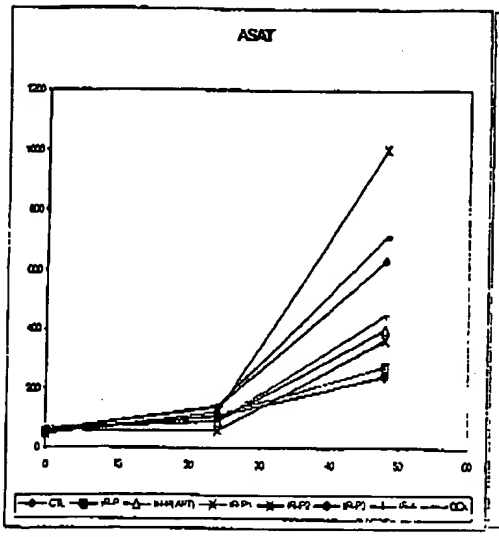


Figure 61

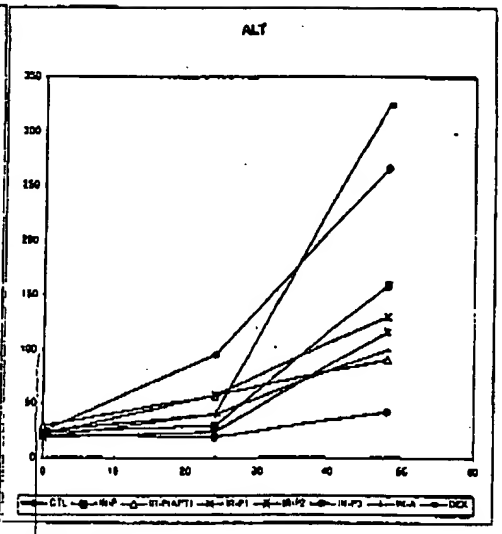


Figure 60

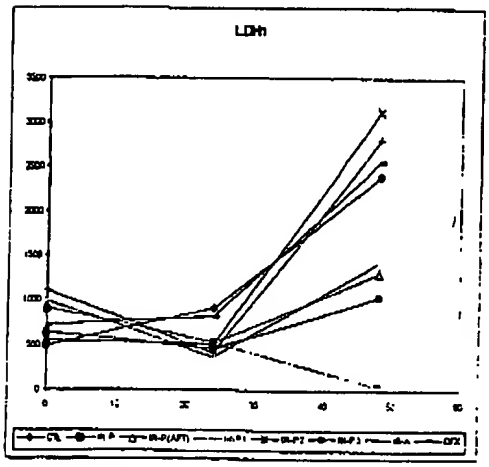


Figure 62

(figure 60-62) shows that mice treated with IR-A, IR-P and its fraction IR-P1, IR-P2, IR-P3 had relatively lower level of ALT, LDH1, ASAT enzymes present in the plasma as compare to control and dexamethasone treated mice. These enzymes are present in higher concentration in blood during shock due to organ damage, so these result are consistant with our surviving results (figure 58).

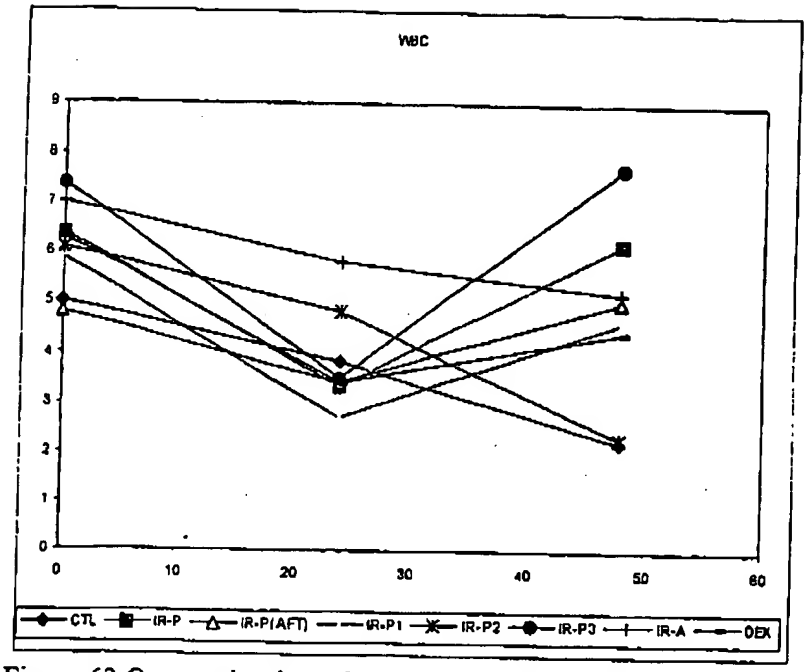


Figure 63 Our results show that mice treated with IR-A, IR-P and its fractions have moderate to normal level of WBC at t=48 hours then the control and dexamethasone treated mice, suggesting less inflammatory responses in IR treated mice.

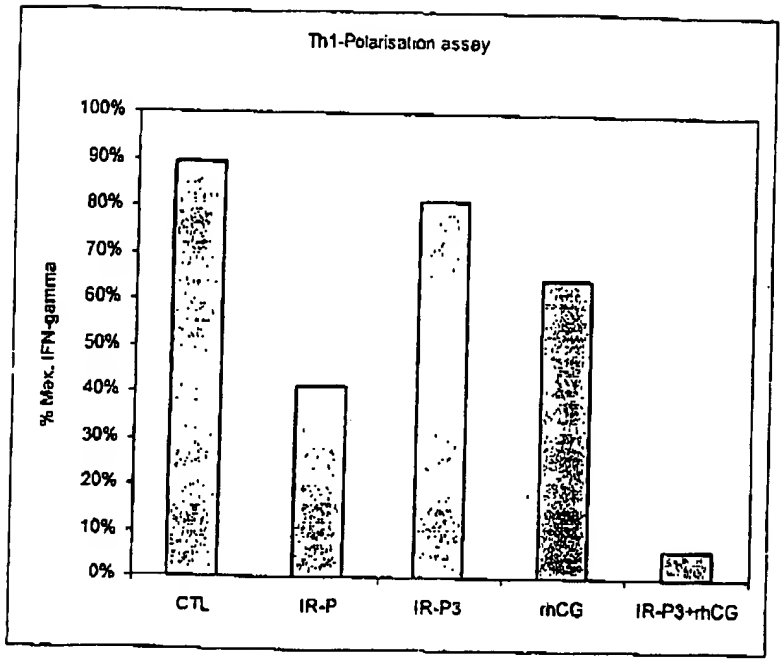


Figure 64 shows inhibition of IFN-gamma production in Th1 polarisation assay of CD4+ cells isolated from IR-P and rhCG in combination with IR-P3 treated NOD mice, while moderate inhibition was found in Th1 polarisation by rhCG and IR-P3 alone. This shows that in NOD mice treated with rhCG in combination with IR-P3 give massive inhibition of Th1 outgrowth. Which suggests that IR-P3 fraction needs rhCG for it maximal inhibition of Th1 subsets.

NOD/LTJ INVIVO TREATMENT (ANTI-CD3 STIMULATION)

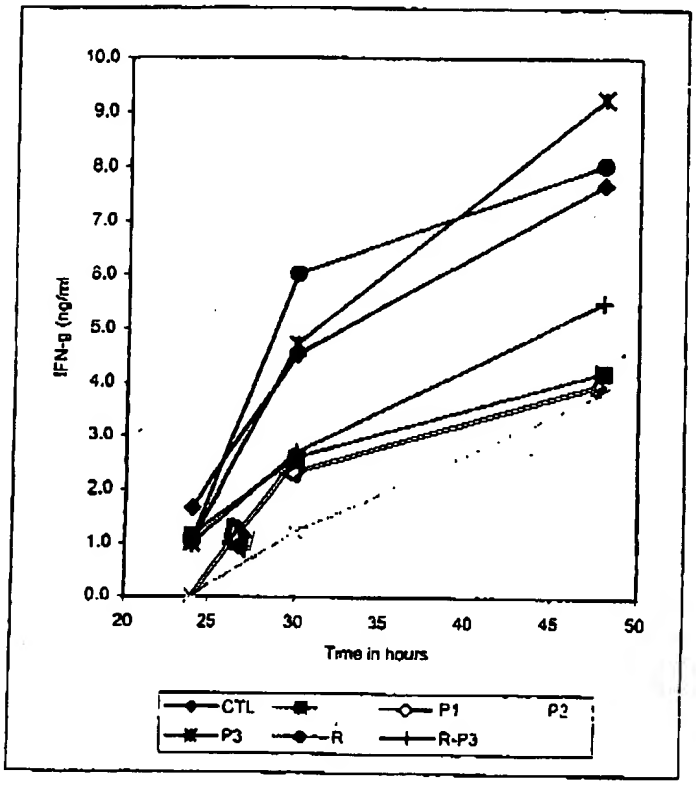


Figure 65

FOR MORE DETAILS, SEE DOCUMENT

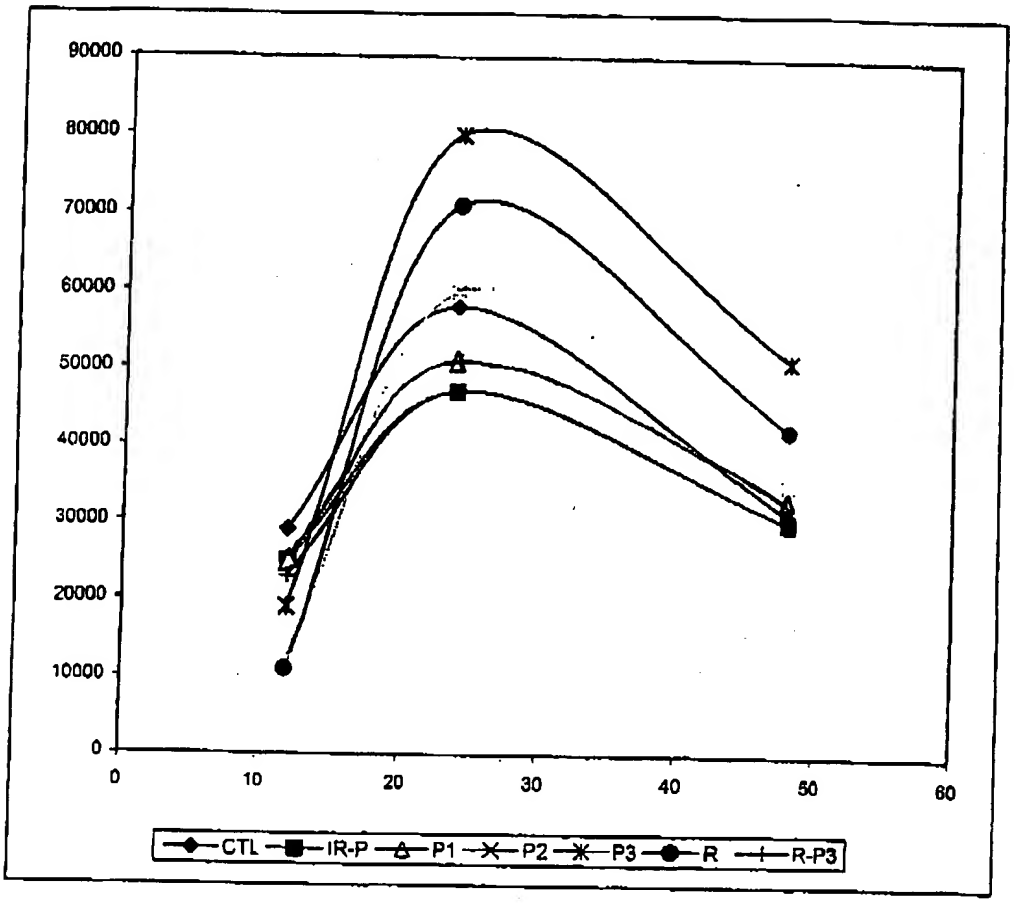


Figure 66

FOR MORE DETAILS, SEE DOCUMENT

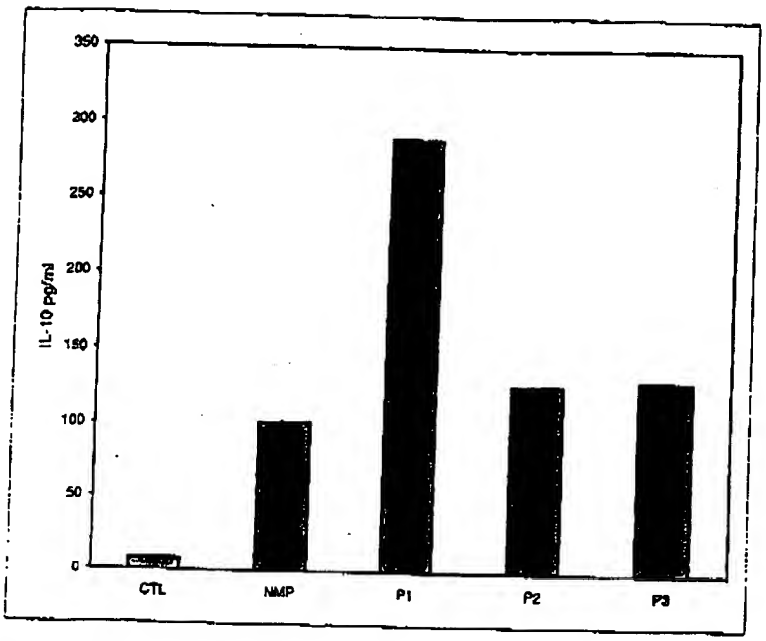


Figure 67 shows that IR-P and its fractions promote IL-10 production of anti-CD3 stimulated spleen cells from treated NOD mice as compare to PBS treated mice.

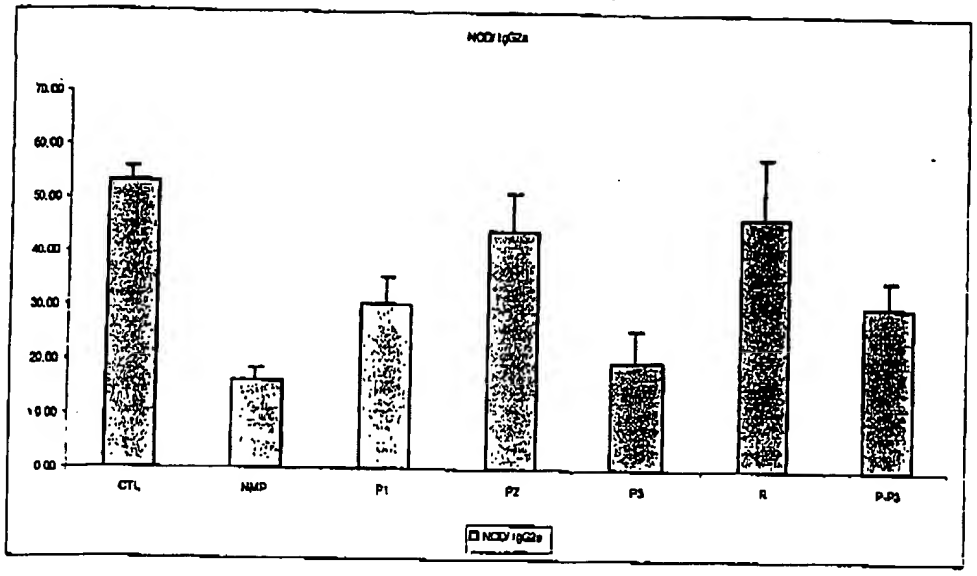


Figure 68 shows that IgG2a production is not inhibited by IR-P2 and rhCG in vivo treatment, while IR-P, IR-P1, IR-P3 and rhCG in combination with IR-P3 inhibit IgG2a production.

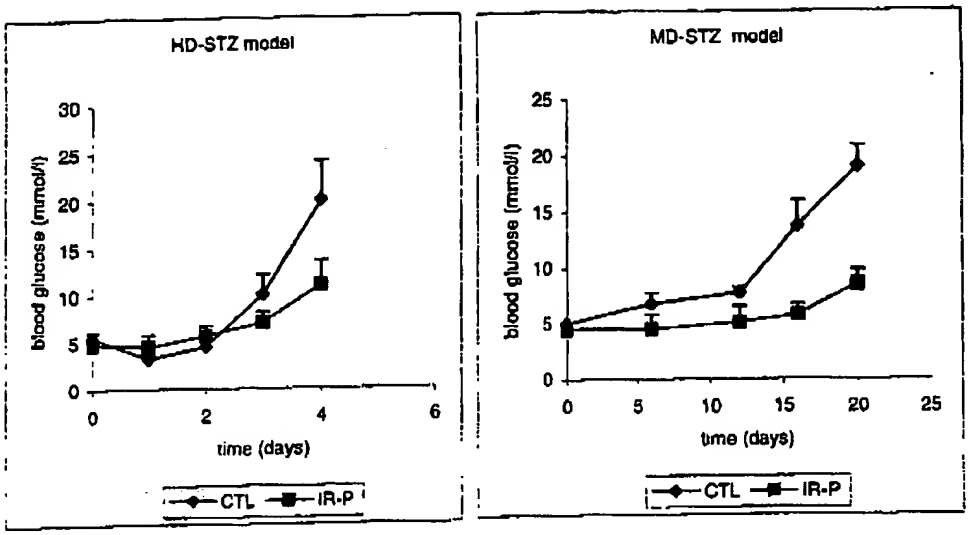


Figure 70

Figure 69

Figure 69 and 70 shows that IR-P treatment is able to delay the induction of diabetes in both model, HD-STZ as well as MD-STZ.

Figure 71

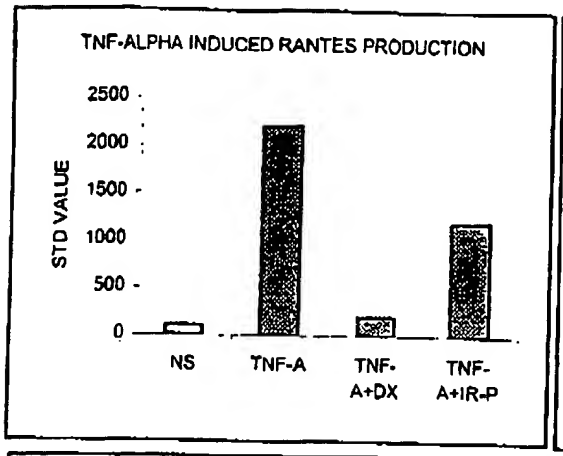
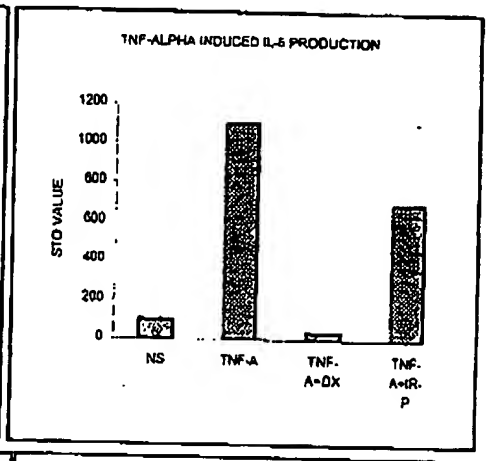


Figure 72



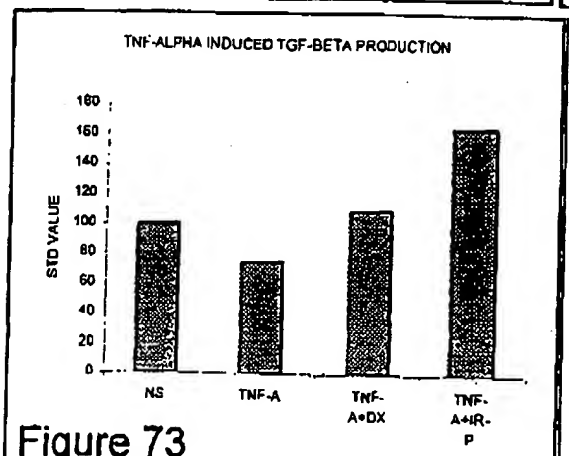


Figure 73

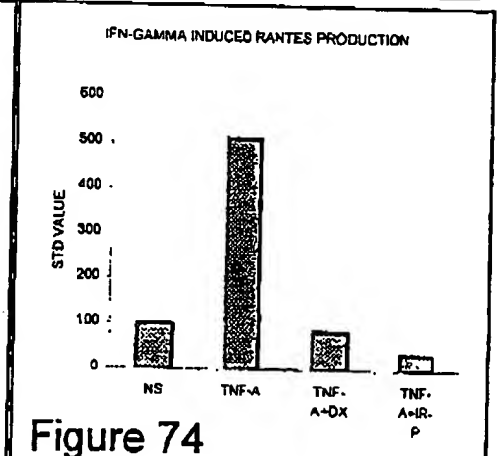


Figure 74

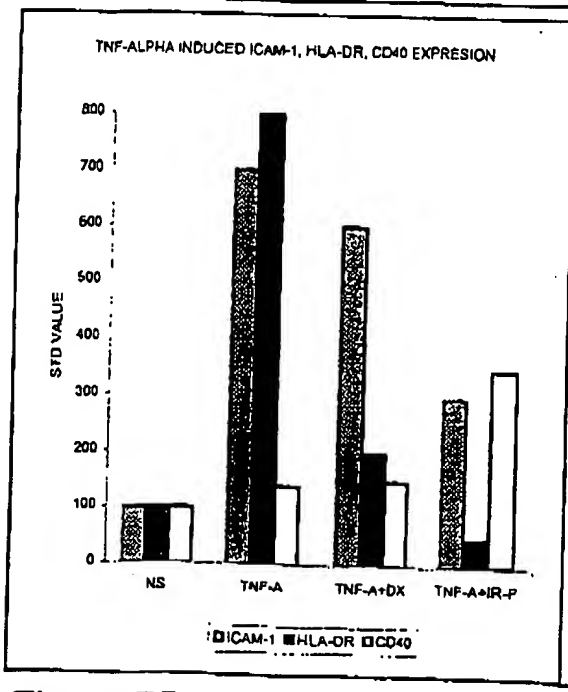
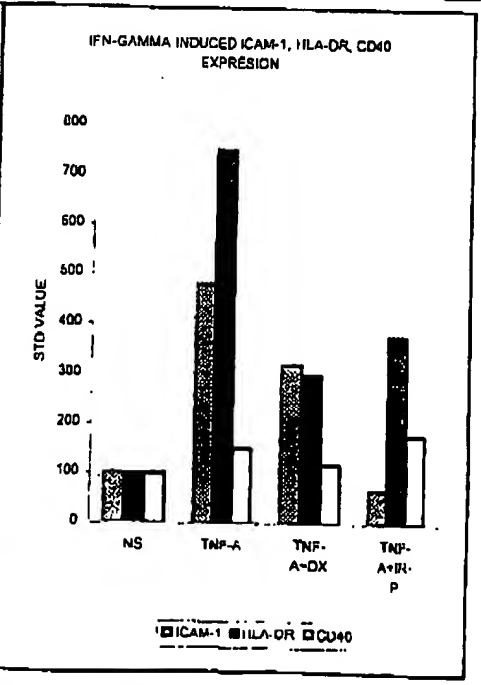


Figure 75



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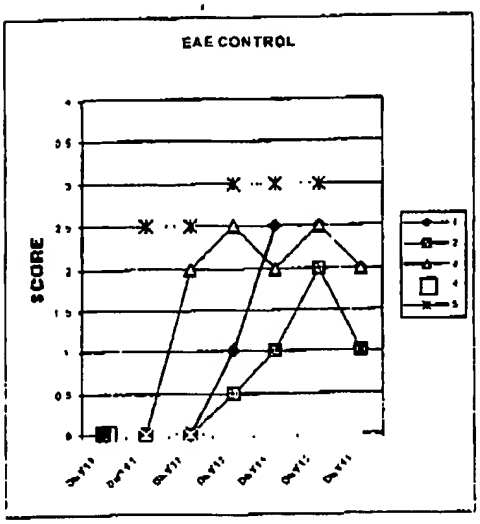


Figure 77

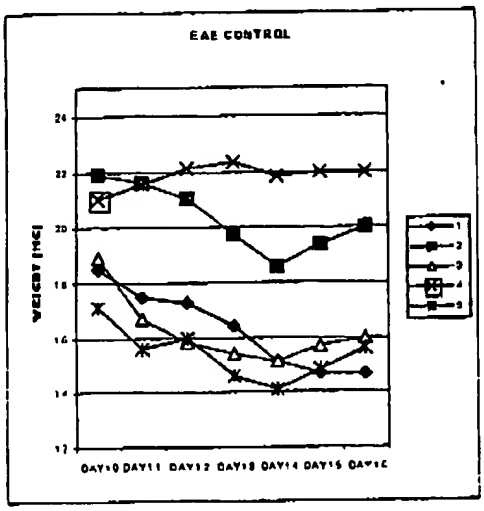


figure 78

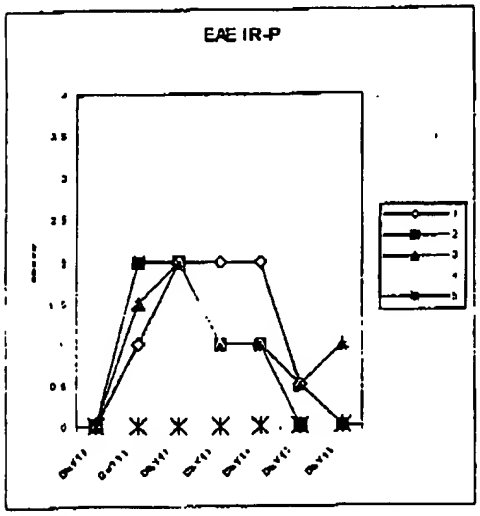


Figure 79

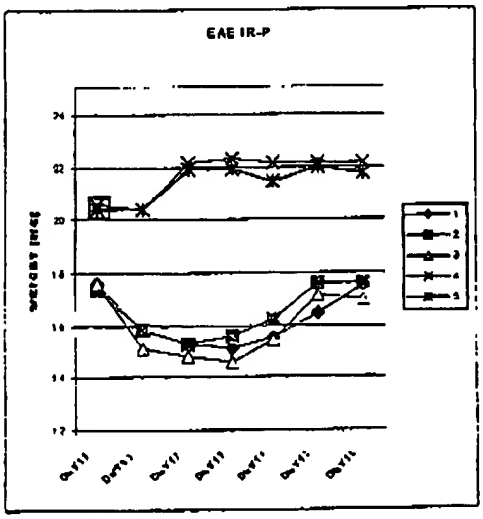
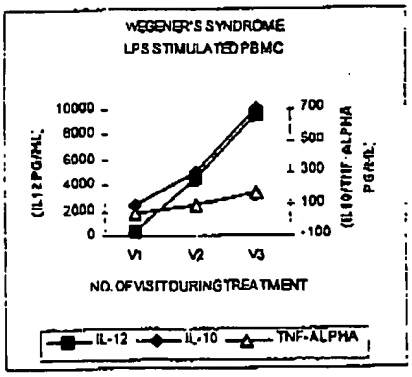


Figure 80

Figure 81

	Before Tx	during Tx	end Tx	Normal (X 10e9
Lymphocytes	0.59	0.75	1.56	1.5 - 4.0
T cell	0.57	0.72	1.48	0.9 - 2.8
CD4	0.24	0.26	0.59	0.5 - 1.7
CD8	0.31	0.41	0.23	0.3 - 0.8
B-cell	0.01	0.01	0.01	0.1 - 0.3

Figure (82a)



(82b)

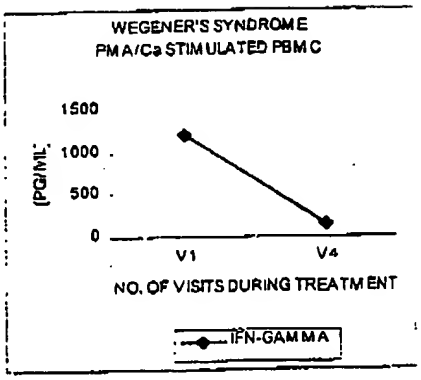


Figure 83

	Before Tx	during Tx	end Tx	Normal (X 10e9
Lymphocytes	2.87	2.06	1.22	1.5 - 4.0
T cell	2.35	1.59	1.02	0.9 - 2.8
CD4	1.95	1.26	0.82	0.5 - 1.7
CD8	0.49	0.37	0.18	0.3 - 0.8
B-cell	0.33	0.19	0.14	0.1 - 0.3

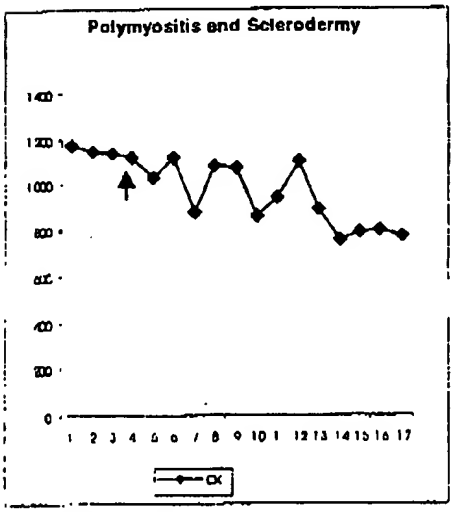


Figure 84

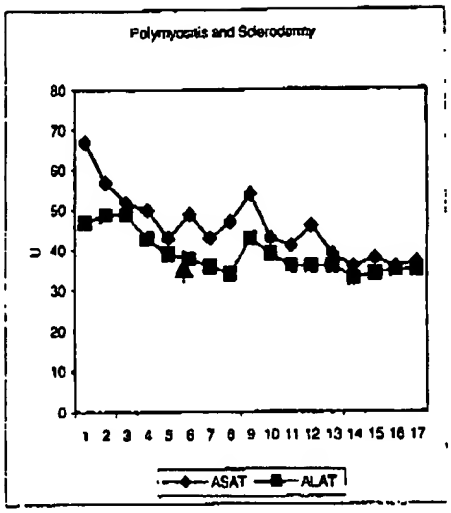


Figure 85

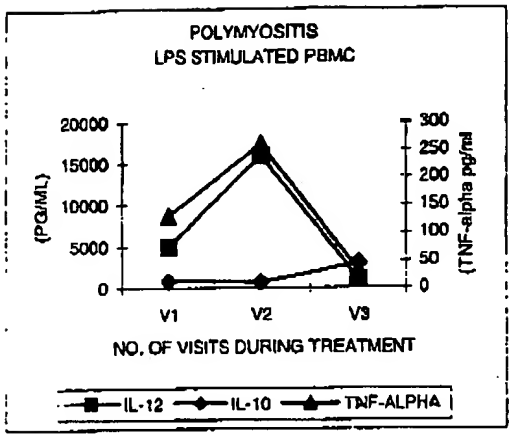

POLYMYOSITIS
LPS STIMULATED PBMC
20000
15000
10000
5000
0
(PG/ML)
300
250
200
150
100
50
0
(TNF-alpha pg/ml
V1
V2
V3
NO. OF VISITS DURING TREATMENT
IL-12
IL-10
TNF-ALPHA

Figure 86

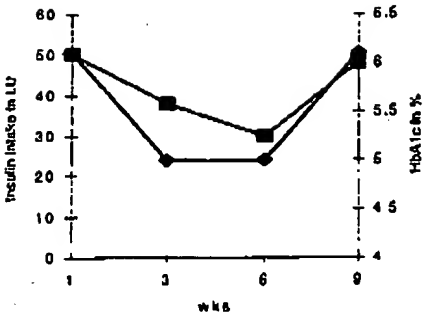


Figure 87

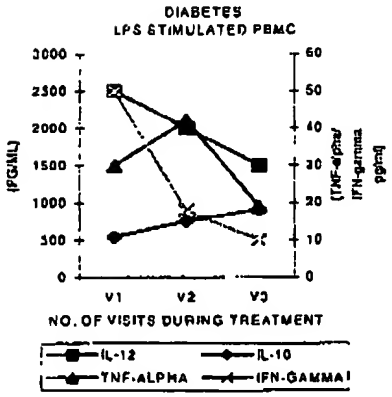


Figure 88

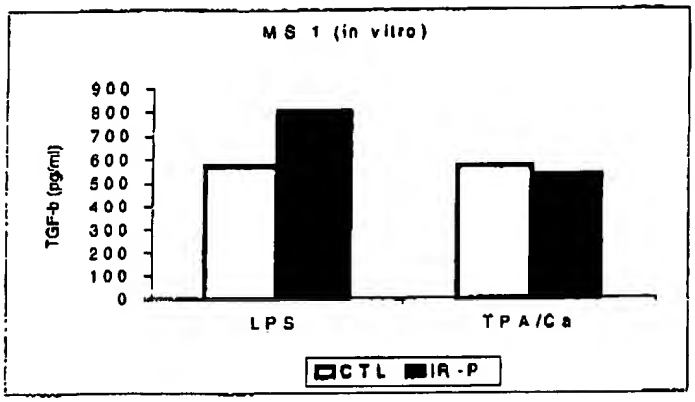


Figure 89

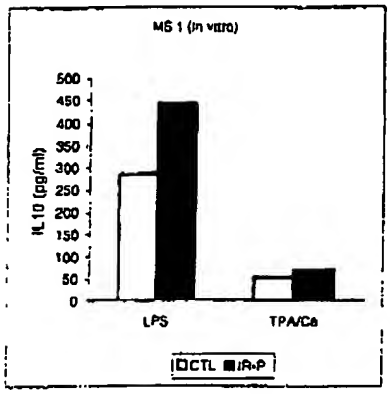


Figure 90

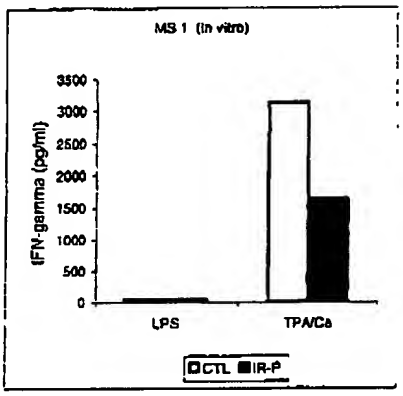


Figure 91

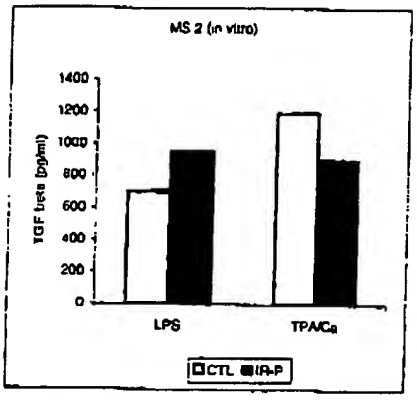


Figure 92

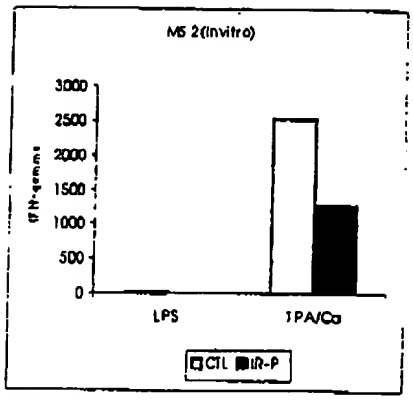


Figure 93

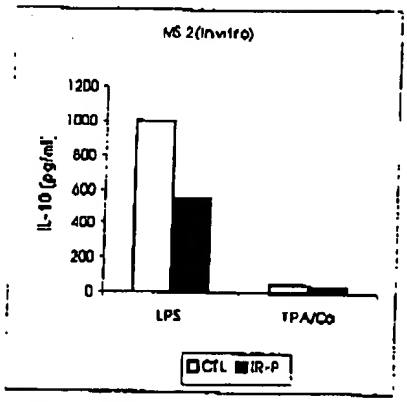


Figure 94

Figure 96

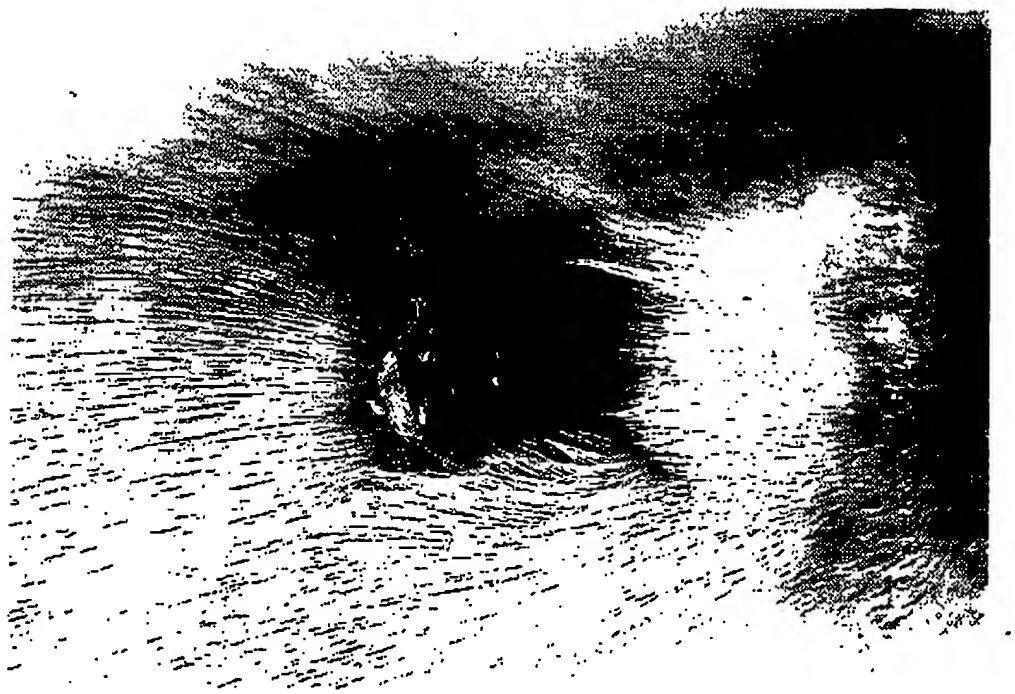


Figure 95

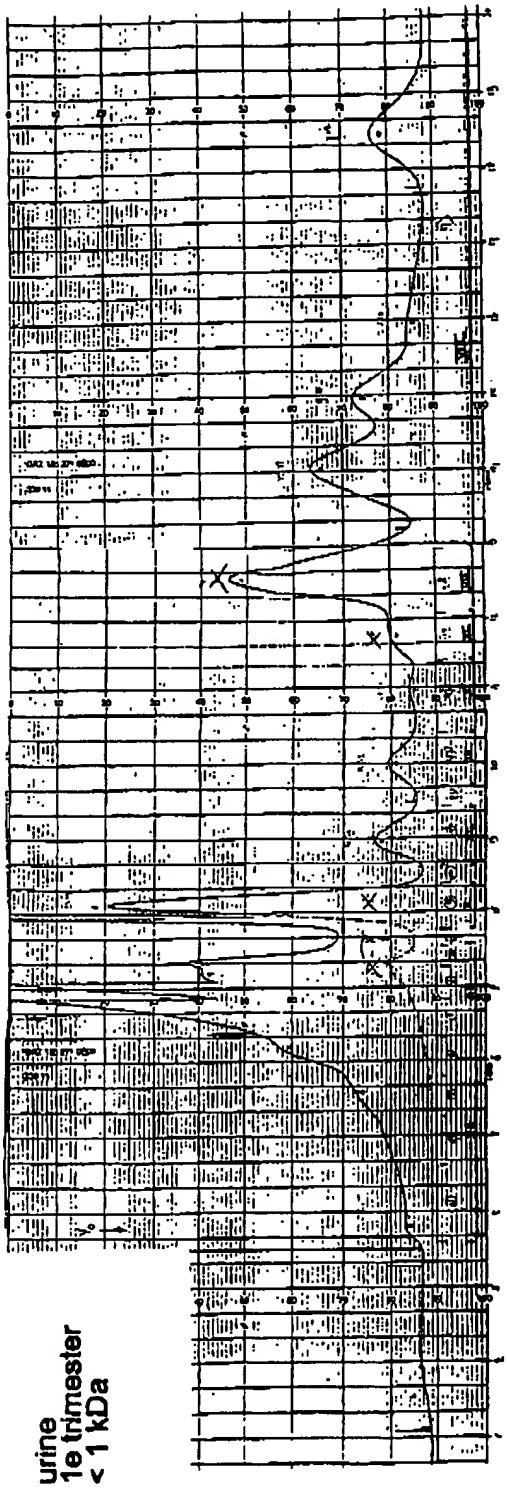


Figure 97

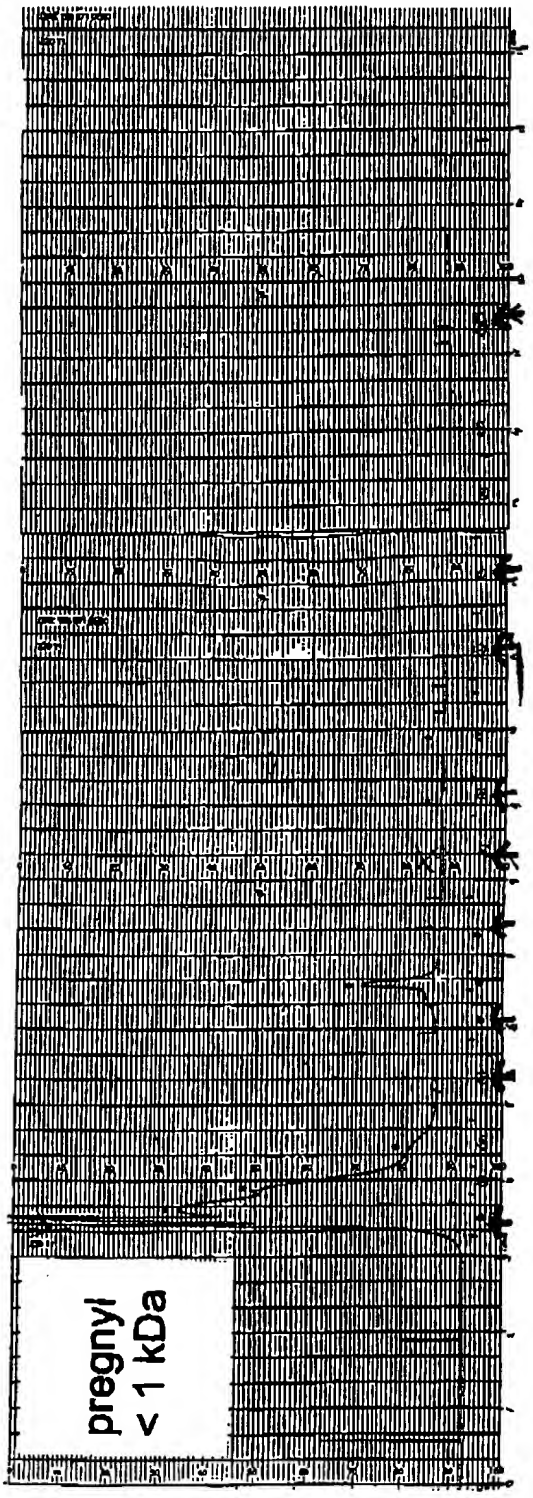


Figure 98

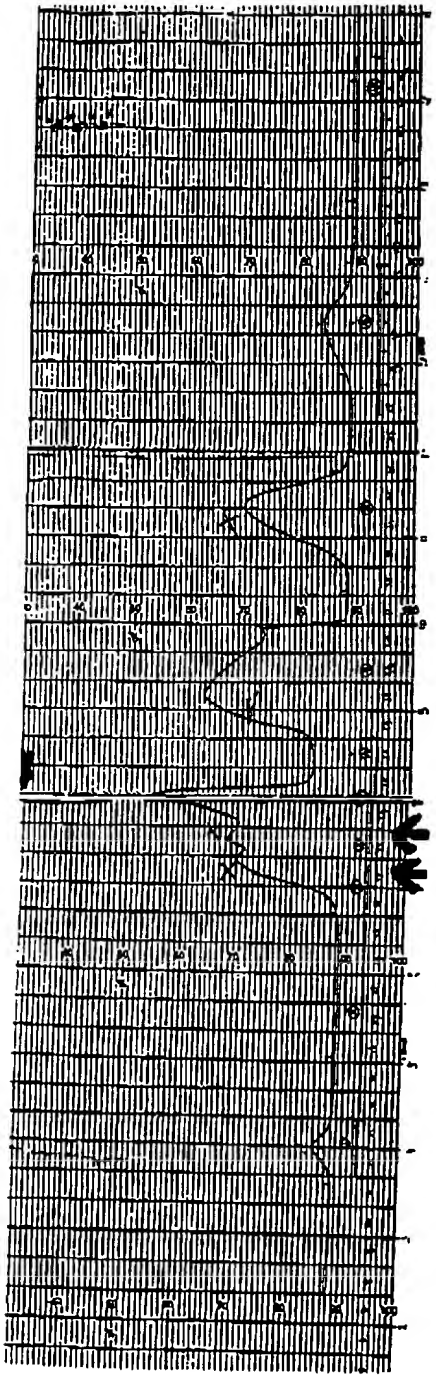


Figure 99

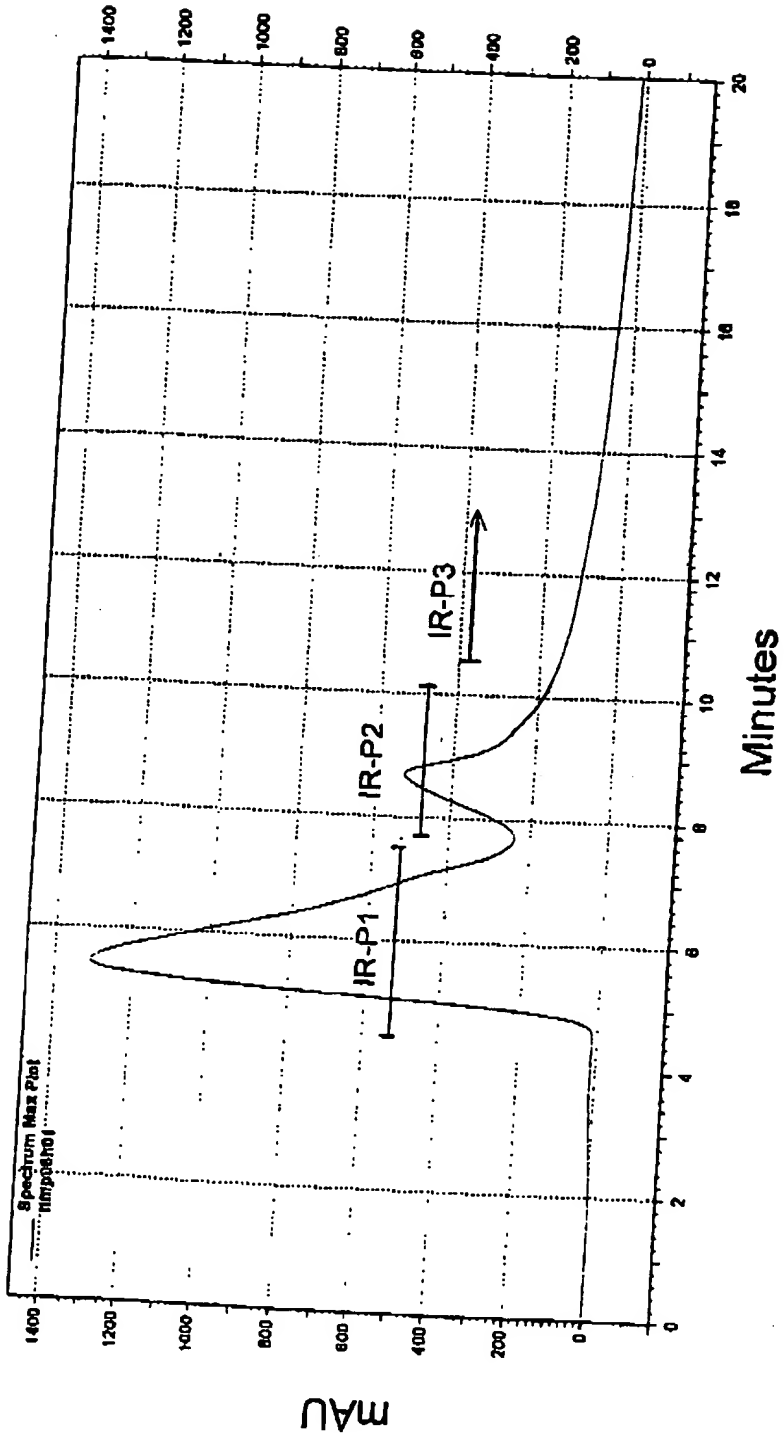
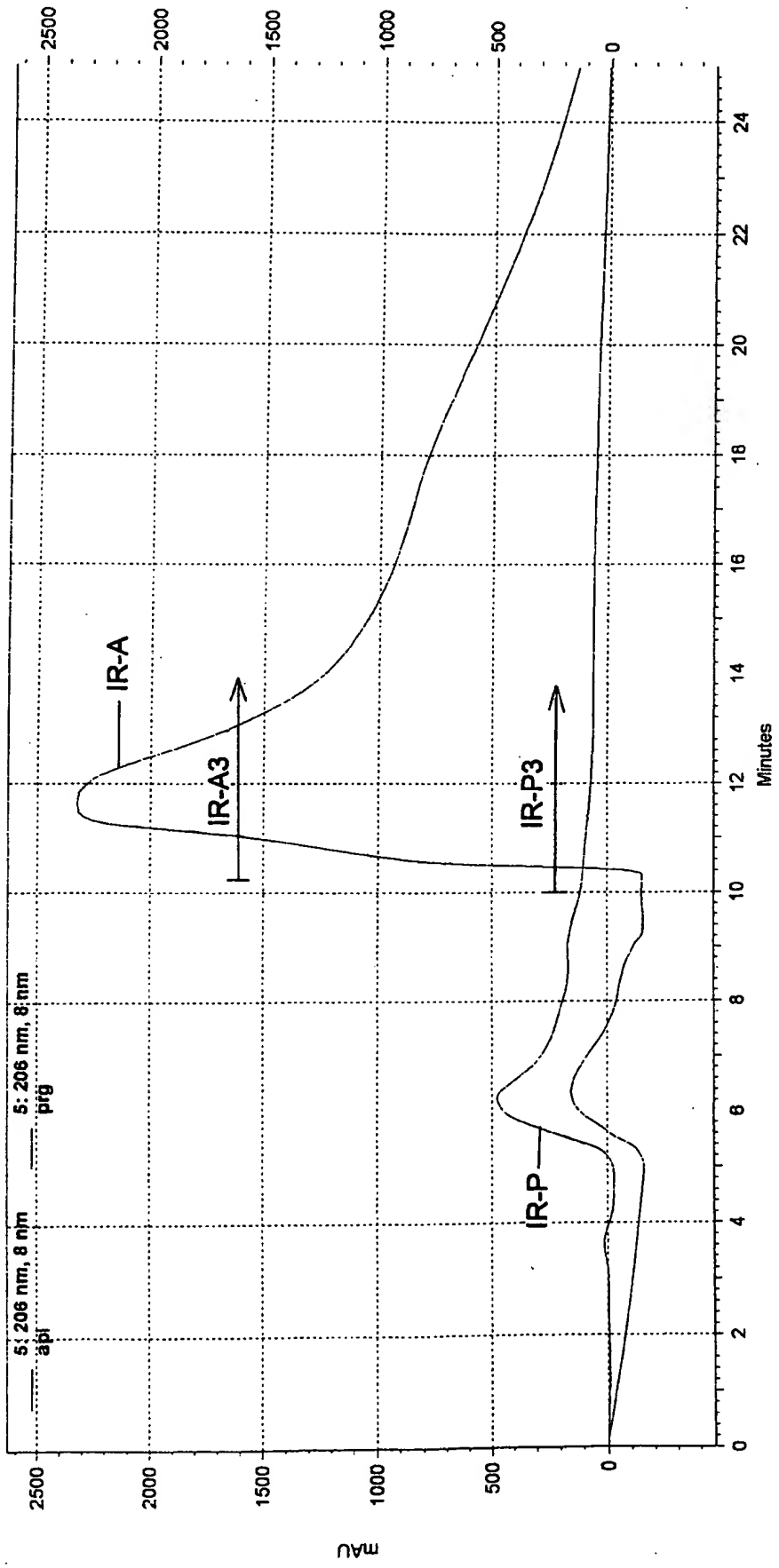


Figure 100



GPC 60 Å

Figure 101

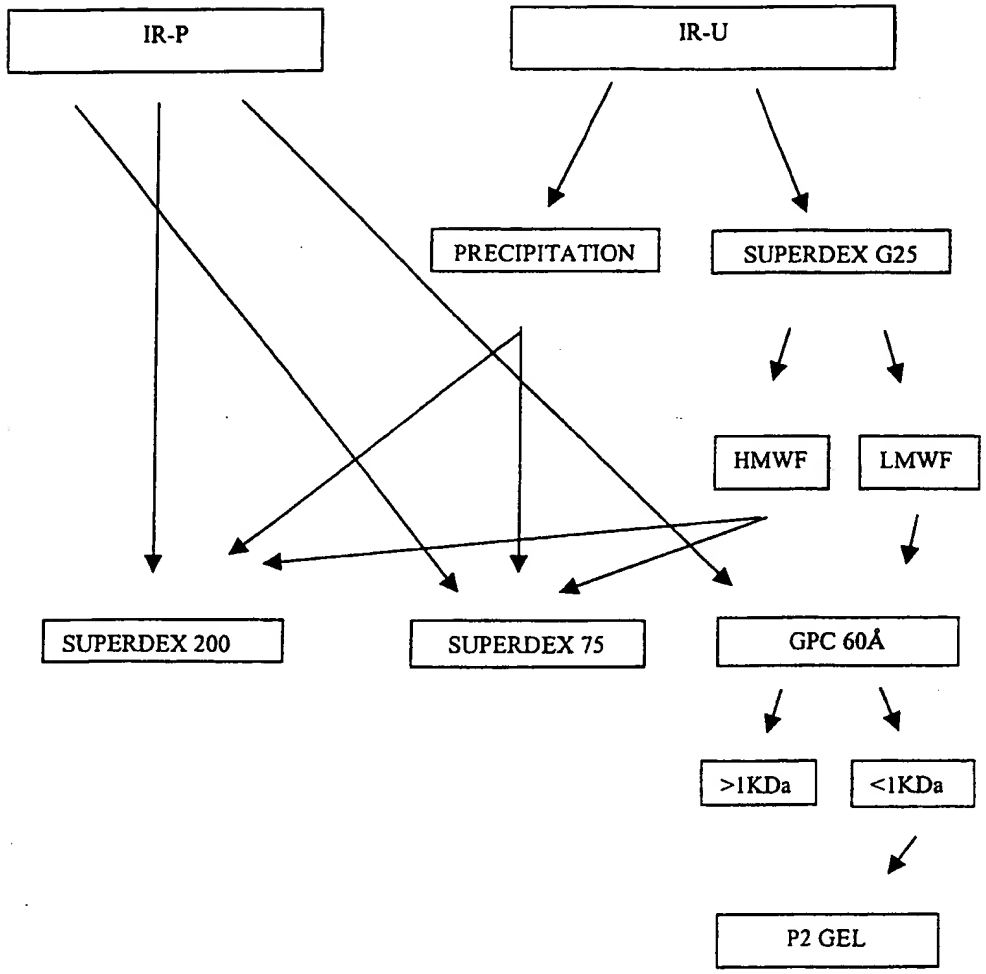

IR-P
IR-U
PRECIPITATION
SUPERDEX G25
HMWF
LMWF
SUPERDEX 200
SUPERDEX 75
GPC 60Å
>1KDa
<1KDa
P2 GEL

Figure 102